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Abstract

A target screening of ca. 800 compounds was performed on water samples taken over a 5-month period in three small contrasting catchments close to Leipzig and Dresden, Germany (Lockwitzbach, Geberbach, and Launzige). The main goals were to determine which compounds classes and sources contribute to the micropollutant patterns, how large is the ecotoxicological pressure of the micropollutants, and to what extend do discharge events effect these results. On-site large-volume solid phase extraction (LVSPE) devices were used for sample collection. Samples were analysed using liquid chromatography-high-resolution mass spectrometry and Toxic Units (TUs; ratio of measured and effect concentration of a compound) were calculated for algae, crustaceans and fish using experimental or predicted toxicity data. A wide variety of compounds were detected, with some such as the benzothiazoles, metazachlor and its derivatives, and lauramidopropylbetaine appearing at up to the µg L^{-1} range. The dominating compound classes typically coincided with land-use, as polymer additives resulting from traffic-runoff dominated in the highly urbanised Geberbach, whereas pesticides were highest in the more rural Launzige. Rain events influenced pollutant compositions to varying degrees between the three catchments. The Geberbach was the most impacted, with the highest mean concentrations doubling and a large increase in polymer additives being detected. Risk assessment using TUs found an increase in risk across all catchments during rain events, however this varied greatly from a 2-fold increase to a whole order of magnitude increase. The risk threshold was exceeded in all cases during rain events and in some cases during baseline conditions. For crustaceans the risk was exceeded in all samples. Pesticides and biocides contributed the most to the total toxicity across all catchments, in particular quaternary ammonium compounds (QACs) (for all species), herbicides (for algae), and insecticides (for crustaceans). Future efforts to improve ecosystem health in these catchments should therefore prioritise reducing the quantities of these compounds reaching the streams.

Zusammenfassung

Ein gezieltes Screening von ca. 800 Verbindungen wurde an Wasserproben durchgeführt, die über einen Zeitraum von 5 Monaten in drei kleinen, kontrastreichen Einzugsgebieten in der Nähe von Leipzig und Dresden, Deutschland, entnommen wurden (Lockwitzbach, Geberbach und Launzige). Die Hauptziele waren zu bestimmen, welche Verbindungsklassen und Quellen zu den Mikroverunreinigungsmustern beitragen, wie groß die ökotoxikologische Belastung durch die Mikroverunreinigungen ist und inwieweit Einleitungsereignisse diese Ergebnisse beeinflussen. Für die Probenahme vor Ort wurden Geräte zur großvolumigen Festphasenextraktion (LVSPE) verwendet. Die Proben wurden mit Flüssigchromatographie und hochauflösender Massenspektrometrie analysiert, und die Toxic Units (TUs; Verhältnis zwischen gemessener und effektiver Konzentration einer Verbindung) wurden für Algen, Krebstiere und Fische anhand experimenteller oder vorhergesagter Toxizitätsdaten berechnet. Es wurde eine Vielzahl von Verbindungen nachgewiesen, von denen einige, wie die Benzothiazole, Metazachlor und seine Derivate sowie Lauramidopropylbetain, in Konzentrationen von bis zu µg L^{-1} vorkamen. Die vorherrschenden Verbindungsklassen stimmten in der Regel mit der Flächennutzung überein, da die aus dem Verkehrsabfluss stammenden Polymeradditive im stark verstädterten Geberbach dominierten, während die Pestizide im ländlicheren Launzige am häufigsten vorkamen. Regenereignisse beeinflussten die Zusammensetzung der Schadstoffe in den drei Einzugsgebieten unterschiedlich stark. Der Geberbach war am stärksten betroffen, wobei sich die höchsten mittleren Konzentrationen verdoppelten und ein starker Anstieg der Polymeradditive festgestellt wurde. Bei der Risikobewertung mit Hilfe von TUs wurde in allen Einzugsgebieten ein Anstieg des Risikos bei Regenereignissen festgestellt, der jedoch sehr unterschiedlich ausfiel und von einem Anstieg um das Zweifache bis zu einer ganzen Größenordnung reichte. Die Risikoschwelle wurde in allen Fällen bei Regenereignissen und in einigen Fällen bei den Ausgangsbedingungen überschritten. Bei Krustentieren wurde das Risiko in allen Proben überschritten. Pestizide und Biozide trugen in allen Einzugsgebieten am meisten zur Gesamttoxizität bei, insbesondere quartäre Ammoniumverbindungen (QACs) (für alle Arten), Herbizide (für Algen) und Insektizide (für Krebstiere). Künftige Bemühungen zur Verbesserung des Zustands der Ökosysteme in diesen Einzugsgebieten sollten daher vorrangig auf die Verringerung der Mengen dieser Verbindungen abzielen, die in die Flüsse gelangen.

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1. Introduction

1.1 Chemical production and release

The use of chemicals, many of which are novel synthetic compounds, in everyday life has been increasing rapidly over the last century (Murmann, 2003). It is difficult to imagine life without them and many are now seen as a necessity for the proper functioning of almost every aspect of modern society. Pharmaceuticals have been vital for curing and preventing many diseases as well as alleviating symptoms of those living with incurable conditions and improving the general well-being of humanity. Pesticides have been crucial for food production which has supported the increasing population. Industrial chemicals are crucial for optimising the function of almost all daily items and products, as more than 95% of all manufactured goods rely on chemistry (Wang et al, 2020).

The increasing production of anthropogenic chemicals is of great concern to scientists and 'novel entities' is one of the nine so called planetary boundaries (Persson et al., 2022; Rockström et al., 2009; Naidu et al., 2021; Wang et al., 2020; Yang et al., 2022). The planetary boundaries define safe operating spaces for each system or process which must not be overstepped to maintain a state of stability on earth. The boundaries are values for variables for which a threshold exists where if the value goes over the threshold, it is likely to destabilise that process. Many of the boundaries are interconnected, for example climate change can impact loss of biodiversity (Meyer et al., 2022; Bellard et al., 2012). The true positions of many thresholds are unknown due to large uncertainties (Rockström et al., 2009). Recently, it has been highlighted that the production of anthropogenic chemicals and introduction to the market cannot be balanced by the current status of global risk assessments and monitoring, and therefore some experts consider this boundary to have already been overstepped (Persson et al., 2022).

The heavy use of chemicals has resulted in micropollutants being found ubiquitously in the environment, even in remote areas far away from the point of use due to various transport processes (Gruber, 2018; Naidu et al., 2021; Evangeliou et al., 2020). Micropollutants can be defined as anthropogenic chemicals that occur in the environment well above the natural

background level due to human activities, but with concentrations remaining up to the microgram per litre range (Stamm et al., 2016). Many micropollutants are so called 'emerging compounds' which the United States - Environmental Protection Agency (USEPA) defines as new chemicals without regulatory status, who's effects on the environment and human health are poorly understood or unknown (Deblonde et al., 2011). Micropollutants can end up in surface waters (e.g., streams, rivers, lakes, and ponds) via various pathways which depend on the specific application such as wastewater, both treated and untreated, and surface runoff from agriculture and traffic. Furthermore, micropollutant dynamics in surface waters are influenced by rain events, which are predicted to become increasingly variable due to climate change.

1.2 Effects of chemicals on ecosystems

Biodiversity is another one of the nine planetary boundaries which must not be overstepped (Rockström et al., 2009). The rapid loss of biodiversity in the last few centuries is of great concern and many scientists have stated that we are in the middle of a sixth mass extinction event (Barnosky et al., 2011; Dirzo et al., 2022). There are many drivers for biodiversity loss but one of which is anthropogenic chemicals reaching the environment and the adverse effects of chemicals on ecosystems is well documented (Godfray et al., 2019; Windsor et al., 2018; Nilsen et al., 2019). Of particular concern are micropollutants which are classified as persistent, bioaccumulative, and toxic (PBT). PBT compounds are very resistant to degradation and therefore persist in the environment for a long time meaning they are more likely to eventually encounter organisms. Their bioaccumulative nature allows them to concentrate in organisms to toxic concentrations and could in-turn effect the higher species which consume them. According to the European Chemicals Agency (ECHA), "a 'Safe' concentration in the environment cannot be established using the methods currently available" (ECHA, 2023a) Another group of concern are persistent, mobile, and toxic (PMT) compounds, whereby the mobile nature of these pollutants allows them to travel over long distances, impacting potentially ecosystems far away from the point of release. Many micropollutants such as pharmaceuticals, pesticides, biocides, personal care products are designed to be biologically active; this trait can often lead them to be easily taken up by organisms, where they might exert adverse effects.

The issue of toxicity is very complex. Many micropollutants are acutely toxic above certain concentrations and this is relatively easy to evaluate using current toxicological assays, however chronic toxicity is more likely to occur due to long term exposure and there is a lack of knowledge on the middle to long term effects of micropollutants on ecosystems (Sousa et al., 2018; Gavrilescu et al., 2015; Deblonde et al., 2015). As previously stated, micropollutants are typically found in the environment at trace concentrations, however the constant exposure to these compounds can still induce toxic effects over the lifetime of an organism (Gavrilescu et al., 2015). When looking at complex mixtures in the environment it does not suffice to assess the individual toxicities of compounds but rather the mixture toxicity must be considered (Altenburger et al., 2004; aus der Beek et al., 2016; Carvalho et al., 2014). Therefore, environmental monitoring programs should assess the potential for toxic effects looking at the combination of micropollutants in streams as a whole.

1.3 Regulation of chemicals

In 2001 the Stockholm Convention on persistent organic pollutants (POPs) was signed and requires its parties to take measures to eliminate or reduce the release of POPs into the environment. Originally there were 12 chemicals banned or restricted under Stockholm Convention, however there is a review committee and since then more compounds have been added for a total of at least 36 chemicals (UNEP, 2020). Due to their persistency and long-range transport, no single state can protect society and the environment from the hazardous effects of POPs, and as of 2022 there are 186 states which have signed the convention. Other than this there are currently no other global legislations which require states to protect people or the environment from hazardous organic chemicals. The World Health Organisation defines guideline values and health-based values for 89 organic parameters, but these are not mandatory for any states to follow (WHO, 2021).

Within the European Union (EU) the Water Framework Directive (WFD) is the legislation responsible for protecting water for human consumption and therefore surface waters. It dictates that member states must implement policies for whole river basins and construct measures which will improve both the chemical and ecological status of the rivers (Liefferink

et al., 2011). The WFD requires Member States to monitor 45 priority substances (PSs) according to given environmental quality standards (EQSs). Under the WFD, Member States have to report any PSs which are found to be above the EQSs and must implement measures to reduce the releases of PSs to the environment. There are an additional 17 Watch List compounds of emerging concern where monitoring data across the whole European Union should be gathered to support future decision making (Sousa et al., 2018, 2019). Member States are allowed to set EQSs higher than those set by the WFD, but they cannot be lower. In Germany, the WFD is implemented by the Federal Water Act (Wasserhaushaltsgesetz, WHG), and has EQSs in line with those set out by the WFD. Additionally, each Member State must identify river basin specific pollutants, Germany has 67 (Weisner et al., 2022).

There is a consensus amongst scientists that regulations are not strong enough (Persson et al., 2022; Kortenkamp and Faust, 2018; K. Mueller et al., 2023). One issue is that they do not cover the right compounds, for example in Germany 75% of the pesticides monitored under the WFD are not approved for use anymore. Conversely, only 6% of pesticides approved for use in Germany are monitored under the WFD (Weisner et al., 2022). Current regulations do not consider the complexity of environmental mixtures and assessing the hazards from individual chemicals fails to accurately consider the impacts from mixture toxicity (Brack et al., 2017, 2022; Drakvik et al., 2020; Scholtz et al., 2022). Enforcement of the regulation is also lagging behind; four countries provide the majority of data and 72% of the data was non qualitative (Heiss and Küster, 2015). 40 European countries (EU and non-EU) have not performed a water monitoring study between 2012-2017. To maintain good water quality standards, it is not only vital to monitor the status of all EU waters but also to report the data to the European Commission to properly inform future action plans and decision making (Sousa et al., 2018).

Globally, regulations vary significantly, however the general trend is that high-income countries have stricter regulations than middle- and low-income countries (Landrigan and Fuller, 2015). The majority of developing countries lack the access to the most modern analytical equipment which hinders the ability to monitor trace level pollutants (Kandie et al., 2020) The effect of little to no regulation in developing countries combined with an increase in chemicals industry in these countries due to cheaper labour expenses is resulting in

significantly higher levels of contamination (de Souza et al., 2020; Wilkinson et al., 2022; Kookana et al., 2014). Allowing this imbalance to continue will be detrimental to all countries, as many micropollutants persist in the environment for a long time after they have been banned and can undergo long-range transport (Lepom et al., 2009; Brack et al., 2022). To ensure the preservation of ecosystems around the world, global policy making regarding chemical pollution needs to consider the impacts of leaving developing countries to fall behind.

2. State of Knowledge, Aims and Hypothesis

2.1 State of knowledge

2.1.1 Current knowledge on occurrence, dynamics, and effects of typical micropollutants

Occurrence

The annual production of chemicals is increasing rapidly with a 50-fold increase since 1950 (Persson et al., 2022). There is estimated to be over 350,000 industrial chemicals globally registered for market use, however the actual number of chemicals on the market may be significantly higher due to various uncertainties in the estimation (Wang et al., 2020). Furthermore, the production and use of chemicals inherently creates unintended by-products, transformation products and impurities which means the actual number of chemicals in the environment and therefore in surface waters is higher (Wang et al., 2020; Persson et al., 2022). In terms of physical quantities, 2.5 x 10^9 tonnes per annum of manufactured or synthetic chemicals are likely released into the environment, and as the production of anthropogenic compounds far outweighs all removal processes, the quantities of chemicals in the environment is largely cumulative (Naidu et al., 2021 and references therein; Cribb, 2014; 2017; 2021). It is known that the presence of organic pollutants in the environment has adverse effects on ecosystems which makes the ever-increasing concentrations of pollutants particularly concerning (Godfray et al., 2019; Windsor et al., 2018; Nilsen et al., 2019). Varying applications affect how often compounds are used and in what quantities, therefore when monitoring pollutant mixture dynamics, it is often useful to examine them as different compound classes.

Pharmaceuticals have been detected in surface waters on every continent on earth and more than 200 pharmaceuticals have been detected in rivers globally (Wilkinson et al., 2022; Hughes et al., 2013). It is predicted that the consumption of pharmaceuticals will increase in the future due to an increasing population, an aging population, the development of new pharmaceuticals, and better access in developing countries (aus der Beek et al., 2016; Hughes et al., 2013). Currently, many pharmaceuticals and their metabolites are not well removed from wastewater and therefore end up in surface waters, detected in the ng to µg L^{-1} range.

An up-to-date and accurate quantification of the global consumption of pesticides is missing from the current literature, however as of 2019 roughly 2 million tonnes of pesticides were used globally per annum with estimations of consumption increasing to 3.5 million tonnes in 2020 (Sharma et al., 2019). It is therefore not surprising that a literature review by de Souza et al. found pesticides in surface waters in North and South America, Europe, Asia, and Africa (de Souza et al., 2020). Pesticides have also been found in surface waters in Australia (Rippy et al., 2017; Anim et al., 2020) and in both the Arctic and Antarctic (Xuan et al., 2023; Zheng et al., 2022; McGovern et al., 2022).

For biocides also the up-to-date quantification of their global production and consumption is missing from the current literature, however 285 biocidal active substances are currently approved for use in the EU, with more undergoing review processes to gain approval (ECHA, 2023b). They have a variety of uses such as pest control, cleaning, and antifouling in building materials and are frequently detected in surface waters globally (Sánchez-Rodríguez et al., 2011; Konstantinou and Albanis, 2004)

Industrial chemicals are a class often mentioned in the literature but there currently is no formal definition. They are considered in the EU under REACH (Registration, Evaluation and Authorisation of Chemicals) and not within another domain (Pesticides, biocides, pharmaceuticals, cosmetics, food additives). Some have well-defined and well-known uses and therefore can be assigned to specific classes such as rubber additives and surfactants. Some are compounds where their specific use is either not clear or covers a broad range of applications therefore resulting in a sort of ‘leftover category’. For this reason, the data

analysis in this report will refer to other use categories for chemicals which in other sources may be classed as industrial chemicals.

Food and beverage chemicals include sweeteners and stimulants, some of which such as sucralose are hardly metabolised (Magnuson et al., 2016) and some of which such as caffeine and nicotine have well-studied metabolic pathways with known metabolites (Senta et al., 2015 and references therein; Garattini, 1993; Hukkanen et al., 2005). Exact consumption data for these compounds is missing, however a 2019 study found that 15% of the German population smokes tobacco daily, and a 2013 study found that the average consumption for caffeine is 2.1 mg kg_{bw}^{-1} day^{-1} (Atzendorf et al., 2019; Lachenmeier et al., 2013). These compounds are removed from wastewater with varying efficiencies and these compounds typically appear in surface waters in ng to µg L^{-1} concentrations (Korekar et al., 2020; Verovšek et al., 2022).

Sources

Urban runoff is a well-known major source of micropollutants in surface waters as rainwater washes anthropogenic chemicals typically used in urban settlements via various pathways into streams and rivers (Göbel et al., 2007; Launay et al., 2016; Mutzner et al., 2022). Urban runoff usually contains many different chemical classes. Compounds which are used in building materials are known to leach into rainwater, this includes flame retardants which are used in insulation materials, biocides which are used frequently as antifouling coatings, and phthalates for which 95% of them are used as plasticisers in PVC which has a variety of uses including roofing and cladding, cable coating, and hoses (Wicke et al., 2021; Björklund, 2010).

A significant portion of urban runoff comprises of micropollutants originating from traffic related uses. Vehicles which run on petrol are known to emit polycyclic aromatic hydrocarbons (PAHs) as a result of the combustion processes in the engine which then typically either remain in the air or sorb strongly to particles and sediments (Nielsen, 1996; Sun et al., 2017). They are also an important source of phthalates as various parts of vehicles including the undercoatings and paint can all contain a variety of phthalates (Björklund, 2010). Another major source of micropollutants from traffic is the release of tire and road wear particles (TRWPs) which is generated by the friction of the tyres on the road. It has been

estimated that 1,327,000 T a^{-1} of TWP are generated in the EU and 133,000 T a^{-1} alone in Germany (Wagner et al., 2018). As well as the TWPs themselves being pollutants as microparticles, other components of the tyres which includes organic micropollutants (table 1) depending on their physiochemical properties can leach into the environment (Wagner et al., 2018; Baensch-Baltruschat et al., 2020; Rauert et al., 2022). All components of the formulation can vary depending on the manufacturer's specifications.

Table 1: Typical composition of tyres, taken from Wagner et al., 2018.

Category	Content [wt%]	Ingredients
Rubber/Elastomer	40-60	poly-butadiene, styrene-butadiene, neoprene isoprene, polysulphide
Reinforcing agent (filler)	20-35	Carbon black, silica, silanes
Process oils	15-12	mineral oils
Textile and Metal net	5-10	
Vulcanisation agent	1-2	ZnO, S, Se, Te, Thiazoles, organic peroxides, nitrocompounds
Additives	5-10	Preservatives (halogenated cyanoalkanes), anti-oxidants (amines, phenols), desiccants (calcium oxides), plasticizers (aromatic and aliphatic esters), processing aids (mineral oils, peptisers)

Agricultural runoff is the largest source of pesticides to surface waters. Pesticides are unique in that they are applied deliberately onto soils i.e., directly into the environment. A significant portion ends up in unintended locations away from the intended place of use. Edge of field losses can be as high as 10% and losses are greatest when rain events occur shortly after application (Schultz. R, 2004). Pesticides can be classified in various ways, according to mode of action, chemical classes, toxicity, and functional groups (Tudi et al., 2021 and reference therein; Garcia et al., 2012). The use of highly persistent organochlorine pesticides is banned worldwide, however some chemical classes of pesticides still in use include organophosphate pesticides, pyrethroids, triazines, and triazoles (Sabzevari and Hofman, 2022).

Wastewater treatment plants (WWTPs) and small-scale treatment systems are designed to remove conventional pollutants such as suspended solids and nutrients which cause eutrophication, they were not designed to remove (emerging) organic pollutants (Adeleye et al., 2022; Guo et al., 2018). They are therefore known to be a significant source of micropollutants in the environment. The removal efficiencies of WWTPs for micropollutants are not properly understood due to a lack of data for individual compounds and variations in microbial communities between plants. Additionally, removal efficiencies vary widely among different plants due to the varying conditions and microbial communities (Larsen et al., 2004). However, as the scale of the issue of micropollutants in the environment has been realised

more studies are focusing on understanding removal efficiencies and creating removal technologies including activated carbon, ozonation, and reverse osmosis as a fourth treatment stage (Margot et al., 2015; Basile et al., 2011; Kosek et al., 2020). There are still many micropollutants which are not held back by these advanced treatment processes such as highly polar compounds, short chain perfluorinated carboxylates and sulfonates, quaternary ammonium compounds, and complexing agents (Reemtsma et al., 2016). Despite this, Finckh et al (2022) found during a risk assessment of wastewater effluent samples that samples which came from WWTPs with a 4th treatment stage had some of the lowest risks of the whole data set.

Domestic wastewater is known to be a significant source of personal care products and detergents which are washed down the drains, plasticisers and fire retardants which leach from textiles and household materials, and pharmaceuticals and their metabolites which are excreted in urine and faeces (Margot et al., 2015; Luo et al., 2014). Hospital wastewater is known to be a significant source of pharmaceuticals in wastewater due to their high consumption (Sörengård et al., 2019). Depending on the sewer system in the area untreated wastewater can reach surface waters during heavy rain events and it has been shown that, despite WWTPs not being optimised for anthropogenic micropollutants, untreated wastewaters have significantly higher concentrations of micropollutants (Phillips et al., 2012; Phillips and Chalmers, 2009; Weyrauch et al., 2010).

Pathways and micropollutant dynamics

Different compounds appear in surface waters in varying concentrations over time and across varying land uses (Wicke et al., 2021; Gooré Bi et al., 2015). This is due to a wide variety of physical factors such as the intensity and frequency of rain events and the catchment characteristics but also anthropogenic factors such as compound application (Spahr et al., 2020). There are various pathways that micropollutants can take to reach surface waters (figure 1). Some pathways lead to a diffuse source of micropollutants to surface waters such as urban and agricultural run-off (Neumann et al., 2002; Müller et al., 2020), and others are a point source of micropollutants such as WWTPs, combined sewer overflows (CSOs), and stormwater outlets (SWOs) (Sörengård et al., 2019).

Different types of sewer systems can have varying effects on micropollutant dynamics in the receiving surface waters. In Europe, the two main wastewater management systems are the separate system and the combined system including modifications, usually a combination of both are used across various catchments (Botturi et al., 2021). The separate system uses a sewage system to take domestic and industrial wastewater to the WWTPs and then uses SWOs to carry rainwater from the mostly impermeable surfaces in urban areas to the nearest surface waters without any form of treatment. Consequently, stormwater in these systems can transport micropollutants resulting from urban, traffic and agricultural runoff directly to the receiving surface waters (Wittmer et al., 2010).

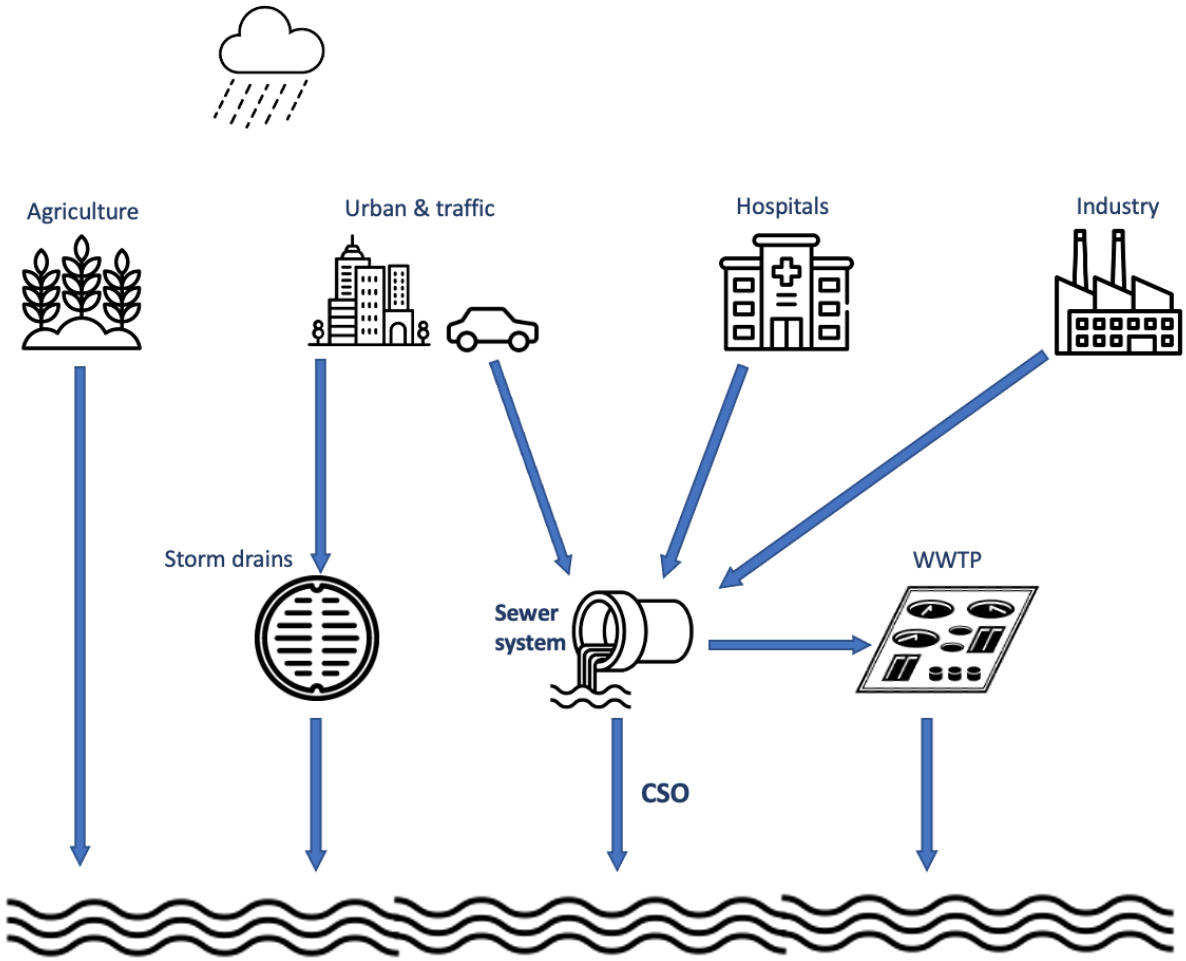

Figure 1: Schematic diagram of sources and pathways of organic pollutants to environmental compartments in a combined sewer system.

Combined systems collect both stormwater and sewage together in one sewer towards the WWTPs. These systems are equipped with combined sewer overflows (CSOs) to prevent WWTPs from exceeding their capacity. In case of heavy rain. The mixture of untreated

wastewater and rainwater will directly flow out into the receiving waters (figure 2). In Europe, approximately 70% of the sewers are combined systems and there are approximately 650,000 CSOs (Botturi et al., 2021). Untreated water from CSOs therefore represents a significant pathway of micropollutants to surface waters (Phillips et al., 2012; Launay et al., 2016; Welker, 2007). In particular, micropollutants with a good removal in WWTPs (>95%) are known to have annual loads which are dominated by CSO input and concentration spikes are seen in rivers during CSO rain events (Weyrauch et al., 2010). Many studies have found that concentration peaks caused by CSO events often take micropollutant concentrations above environmental quality standards (EQS) (Welker, 2007; Mutzner et al., 2022). Variability in concentrations detected between events and sites is high and therefore it is hard to make general statements about micropollutant dynamics affected by CSO inputs without a large number of samples (Mutzner et al., 2020, 2022; Madoux-Humery et al., 2013; Gooré Bi et al., 2015).

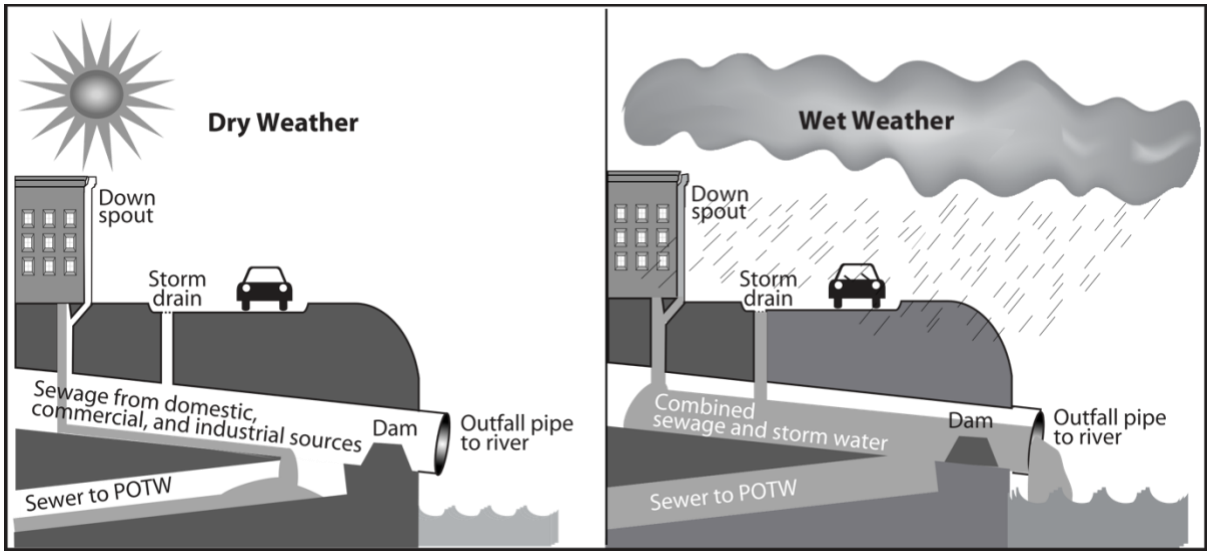

Figure 2: CSOs working in dry and rain events. POTW: publicly owned treatment works (From U.S. Environmental Protection Agency (EPA)).

The pollutant dynamics of any river catchment is also known to be majorly influenced by rain events, both by the input of micropollutants and the change in water volume. Many rain events exhibit a phenomenon known as 'first flush' dynamics which can be described either in terms of concentration or mass. For concentration, the first flush is described as where peak concentrations occur before hydrograph peaks, whereas the definition for mass first flush describes a situation where 60-80% of the pollutant mass is transported by the first 20-30% of runoff volume (Peter et al., 2020). Typically, the more urbanised an area is, the higher

the percentage of stormwater volume that contributes to the surface runoff due to a higher area of impermeable surfaces (Shuster et al., 2005). In small to medium-sized catchments with limited dilution capacity the contribution of urban runoff to the overall pollutant dynamics of the water body is especially significant (Peter et al., 2020). In addition to the surface runoff micropollutants being flushed into surface waters, a study by Gooré Bi et al. (2015) suggested that sediments in sewer pipes deposited during dry periods are remobilised during rain events thereby flushing the particle-bound micropollutants into the receiving waters.

Dilution also plays a role in pollutant dynamics of surface waters. For some micropollutants which are coming only from WWTP effluents the increased volume from the rainwater can cause a dilution of these particular micropollutants in catchments with separate sewer systems. It has also been shown that more dilute runoff is generated by more intense rainfall events which produce a larger water volume (Gooré Bi et al., 2015). As the pollutant dynamics can change very rapidly, the timing of sampling and the temporal resolution between samples are critical factors to consider when characterising pollutant mixtures in surface waters (Lee et al., 2007). Dilution effects in smaller streams and tributaries are significantly lower which means the stress on these ecosystems from the effects of micropollutants here can be higher (Lorenz et al., 2017).

Seasonal variations can also play a role in the pollutant dynamics, as seasonal variations in storm frequency and intensity will affect how often surface pollutants are being washed into surface waters, how often CSOs overflow, and to what degree the pollutants are then diluted. Climate change is however predicted to change geographical precipitations patterns leading to more variability between storms and higher uncertainties, which in turn will be a bigger challenge for risk mitigation projects (Arnone et al., 2018).

Ecological effects

The adverse effects of anthropogenic chemicals on the environment have been long known since the 1960s when the book 'Silent spring' brought attention to the issues of chemical pollution in the environment, specifically pesticides, and the resulting harm on ecosystems and biodiversity loss (Pimentel, 2012). Whilst there are other drivers such as habitat

destruction and climate change, the ever-increasing cocktail of chemicals in the environment has been well documented in numerous studies to be a significant driver for biodiversity loss (Godfray et al., 2019; Windsor et al., 2018; Nilsen et al., 2019; Groh et al., 2022; Bernhardt et al., 2017). Along with novel entities, biodiversity is one of the planetary boundaries which should not be overstepped as it would risk the safety of humanity. Given the importance of functioning ecosystems it is important to properly understand the status of surface waters as important aquatic ecosystems for a great many species to conserve and regenerate them properly and efficiently.

Many of the chemicals being used by society today were designed to be biologically active, for example, pesticides, biocides, and antibiotics are synthesised with the aim of killing or inhibiting the target organism. Therefore, they also have the potential to affect non-target organisms. A particularly problematic group of compounds are the PBT compounds as their persistency means their exposure time for organisms living in habitats polluted by these compounds is high, which could lead to chronic toxicity. They are also bioaccumulative as they are stored in the adipose tissues of the organisms which take them up (La et al., 2013), resulting in a further enrichment along the food chain finally potentially reaching toxic concentrations in top predators (Aravind Kumar et al., 2022). Similarly, persistent, mobile, and toxic (PMT) compounds are of concern due to their mobility, which results from their lack of affinity to soils and sediments which allows them to rapidly distribute in the water cycle and as such travel over great distances (Neuwald et al., 2022).

Another group of pollutants which are of great concern are endocrine disrupting compounds (EDCs). These are compounds which can interfere with the hormone system. This in turn can create harmful effects as the hormone system is responsible for many of the vital functions in the body such as reproduction, growth, and metabolism. Aside from it being suggested that EDCs are a contributing factor to dropping human fertility rates (Karwacka et al., 2019), they have been reported to have critical effects on a wide variety of other species including egg-shell thinning, feminisation of fish and amphibian species, and male rat genital malformations, and many other issues across a variety of species (Guilette, 2006; Hamdhani et al., 2020; Hass et al., 2012; Colborn et al., 1993). This amalgamation of varied adverse

effects is considered to be one of the factors causing population declines across many species (Vos et al., 2000; Godfray et al., 2019).

Many micropollutants found in the environment today are known to be toxic above certain concentrations, however the more insidious issue is the effect of mixture toxicity on ecosystems. It has been known for many decades that combinations of various compounds can bring about toxic effects via various modes of action. The effects of mixture toxicity were originally discovered by pesticide producers who wanted to be more economical in their use. It was discovered that the toxicity of various chemical combinations is not always simply the summation of the toxic effects, but sometimes the toxicity is reduced (antagonistic behaviour) or increased (synergistic behaviour) relative to the additive toxicity. This presents a large challenge particularly for risk assessment (discussed in section 2.1.2). Nagy et al. (2020) found that for 24 out of 36 toxicity studies on pesticide formulations there was an increased toxicity in the pesticide formulation compared to the toxicity of its declared ingredients. Various studies on pharmaceuticals and PCPs have also indicted increased toxicity when combined with other compounds (Schnell et al., 2009; Backhaus et al., 2011; Soler de la Vega et al., 2019), and the same has been shown for a variety of other chemicals (Breitholz et al., 2008; Walter et al., 2002).

2.1.2 Risk assessment

Due to the ever-increasing number and quantities of anthropogenic chemicals being produced, humans and wildlife alike are constantly being exposed to a cocktail of compounds, each of which carries not only its own risk but also the risk associated with that compound in the mixture of which it finds itself. Current risk assessment approaches by regulatory bodies focus on doing a risk assessment of individual chemicals within a mixture, however it has been highlighted by a growing body of evidence that this does not suffice and may lead to an underestimation of the risks presented by mixture (Kortenkamp and Faust, 2018). There are significant knowledge gaps on the toxicological effects of many chemicals on the market and their transformation products or metabolites (Bopp et al., 2019; Fenner et al., 2013; Backhaus and Karlsson, 2014). This raises the question of how best to do a risk assessment for chemical mixtures, as whole mixture data is also very limited, and it is not feasible to test every possible combination of chemicals. There is therefore a need for simplifications in the assessments

which predict exposure and effects, which also highlights the need for holistic mixture risk assessments (MRAs) (Drakvik et al., 2020).

When calculating the expected mixture toxicity there are two fundamental concepts; Concentration Addition (CA) and Independent Action (IA), which are both based on the toxicities and concentrations of individual compounds in a mixture and are both therefore only applicable for well-defined mixtures (Backhaus and Faust, 2012). This is naturally far from the case in the majority of environmental samples. Over the last few decades other methods for assessing mixture toxicity have been developed, often referred to as new approach methodologies (NAMs) which can help account for knowledge gaps and be used at the screening level to help prioritise where to do a full risk assessment. NAMs include *in vitro* methods, Quantitative Structure Activity Relationships (QSAR), Threshold of Toxicological Concern (TTC), read across, toxicokinetic models, and omics (Bopp et al., 2019).

When doing a risk assessment there are different risk metrics which can be used, which each have varying levels of sensitivity and bias towards various compounds. Finckh et al. (2022) assessed the risk of WWTP effluents using three different metrics which are based on different concepts; Risk Quotients (RQs), Hazard Units (HUs), and Toxic Units (TUs) (Equations 1, 2, and 3, respectively), and compared the differences. RQs are calculated by dividing the measured environmental concentration (MEC) with the environmental quality standards (EQS) or predicted no effect concentrations (PNEC). HUs are calculated by dividing the MEC by the concentration where 5% of the species are affected ($HC5_{SSD}$) according to species sensitivity distributions (SSD). TUs are calculated for different biological endpoints by dividing the MEC by experimental effect concentrations (typically EC_{50} or LC_{50} values).

(1) $$RQ_i = \frac{MEC_i}{EQS_i \; or \; PNEC_i}$$

(2) $$HU_i = \frac{MEC_i}{HC5_{SSD,i}(PAF_i{=}0.05)}$$

(3) $$TU_{BQE,i} = \frac{MEC_i}{EC \; or \; LC_{50,BQE,i}}$$

According to Finckh et al. (2022), RQs are most often used in chemicals regulation and implement the precautionary principle by aiming for protection against all impacts on organisms by considering the uncertainty of data. This metric is based on the most conservative assumptions; however, it has a high degree of bias towards pollutants for which no toxicity data exists. The HUs use SSDs based on ecotoxicity data, however data poor compounds are excluded when data is lacking for different species. TUs are calculated using experimental effect concentrations. In cases where experimental toxicity data does not exist, QSAR models can used for a prediction. Each of these metrics can be summed up (assuming CA) for different compounds to a measure of the toxicity of the whole mixture. The use of TUs allows identification of the most relevant compounds in terms of their contribution to the overall toxicity of the mixture (Ginebreda et al., 2014).

2.1.3 On-site large volume solid phase extraction

This study implements the use of on-site large volume solid-phase extraction (LVSPE), a relatively novel but well-established sampling technique first developed and assessed in a study by Schulze et al. (2017). The device allows for an automated and unattended sampling of larger quantities of water over a defined time scale. Often sampling of surface water is done using relatively low frequency grab sampling which provides a spotlight of the contamination at the time of sampling, but no temporal information, while it is well documented that concentrations can vary by over an order of magnitude (Brack et al., 2017; Vasseghian, 2021; Petrie et al., 2015; Spahr et al., 2020). While programmable automatic water samplers can obtain such time-resolved sampling, the prolonged residence time of water in sampling bottles needs a frequent collection of the samples. In contrast, an on-site solid-phase extraction allows a much better stability of the compound on the cartridge over a longer time.

The use of *large volume* SPE offers the possibility for effect-based monitoring using bioassays, without the need to transport large volumes of water to and store in the laboratory (Schultz et al., 2017). While effect-based monitoring was not conducted within this thesis, it was part of the monitoring campaign, making the use of on-site LVSPE a prerequisite for this project. A description of how the device is used is included in section 3.2.

In the pilot study by Schulze et al. (2017) in order to check the recovery efficiency and accuracy 3 replicates of 10 L of pristine natural water spiked with 251 compounds was passed through the device. Of these compounds 249 were recovered, 246 of which were recovered by the HR-X sorbent. The average recovery of the spiked compounds was 88 ± 43% and for the HR-X sorbent 204 of the compounds had a recovery above 50%. The device was then also used at 18 sites in six EU countries, indicating the applicability of LVSPE in the field. The device has since been used in numerous projects at many different sites with success (Finckh et al., 2022; Välitalo et al., 2017; Zhou et al., 2022; Hashmi et al., 2018; Neale et al., 2020; Beckers et al., 2018).

2.2 Aims of the thesis

The aim of this study is to perform an exploratory monitoring of micropollutant mixture composition and dynamics in three contrasting small catchments close to Dresden and Leipzig. To that end, three sub-questions are raised:

How do individual compound classes and sources contribute to the micropollutant patterns in the studied streams? The land uses within the catchments vary from relatively rural to urbanised. In catchments with a predominance of agriculture higher concentrations of pesticides are expected whereas in the more urbanised catchments higher concentrations of traffic related pollutants, while all catchments should see a contribution from domestic wastewater containing pharmaceuticals, personal care and household compounds, and food and beverage compounds.

To what extent do discharge events affect the typical pollutant mixtures found under baseline discharge conditions? It is expected that rain/discharge events will result in an input of untreated wastewater through combined sewer overflows as well as road and surface runoff in the Lockwitzbach and Geberbach catchments, while for the Launzige the impacts on agricultural runoff and on the decentralized, small-scale wastewater inputs are less clear.

How large is the ecotoxicological pressure of micropollutants in these streams and how do discharge events influence this? The Toxic Unit approach (see section 2.1.2) is implemented

here to assess the overall toxicity of the mixtures and to identify the most relevant compounds in terms of their contribution to the toxicity of the mixture in these catchments.

3. Materials and Methods

3.1 Study design and site descriptions

3.1.1 Study design

Three separate stream catchments were chosen for this study, the Geberbach and Lockwitzbach both tributaries to the Elbe in or near Dresden, and the Launzige which is a tributary to the Mulde near Leipzig. Samples were taken over a 5-month period from April-August 2022. To investigate the difference between normal baseline concentrations and rain event-based inputs, both 4-week baseline and rainfall triggered event LVSPE samplers were installed. Due to logistics, it was not possible to put both types of samplers at every site, a list of locations and sampler type is shown in table 2. To investigate the effect of different types of inputs (e.g., agricultural, surface runoff, treated and untreated wastewater) various positions in each catchment were chosen, as described in section 3.1.2. At the Lockwitzbach and Geberbach, no tributaries are present between Lock3 and Lock4 and Geb2 and Geb3, respectively, so that the baseline compositions would not change, and no baseline samplers were installed at the downstream sites. Respectively, it was assumed that event inputs would not be significant at the upstream sites Lock1 and Geb1 and Laun1, so event samplers were placed mostly at downstream sites.

Table 2: List of sites with coordinates and sample type.

Site	Lock 1	Lock 2	Lock 3	Lock 4	Geb 1	Geb 2	Geb 3	Laun 1	Laun 2
Coordinates	50.884062 13.735447	50.956103 13.772467	50.983519 13.800051	51.014011 13.844874	50.990658 13.778646	51.011718 13.797971	51.027813 13.823495	51.300213 12.871149	51.298184 12.819223
Baseline	X	X	X		X			X	X
Event		X	X	X		X	X		X

3.1.2 Site descriptions

Figure 3 shows the locations of the samplers in the Lockwitzbach catchment (area 84 km^2, river length 29 km). There is considerable agricultural use in the upstream part of the catchment along with forests, small villages, and the town of Kreischa, while the downstream section is heavily urbanised. In Kreischa, located between Lock1 and Lock2, there is a large rehabilitation hospital which emits into the communal WWTP. Upstream, several small

WWTPs and locally small-scale on-site wastewater treatment exists. In the heavily urbanised area near Dresden there are various combined sewer overflows and rainwater inlets which result in untreated wastewater and surface runoff input. Here the baseline concentrations from Lock3 will be a proxy for the baseline at Lock4. The Lockwitzbach catchment is part of the Urban observatory of the Chair of Urban Water Management, TU Dresden.

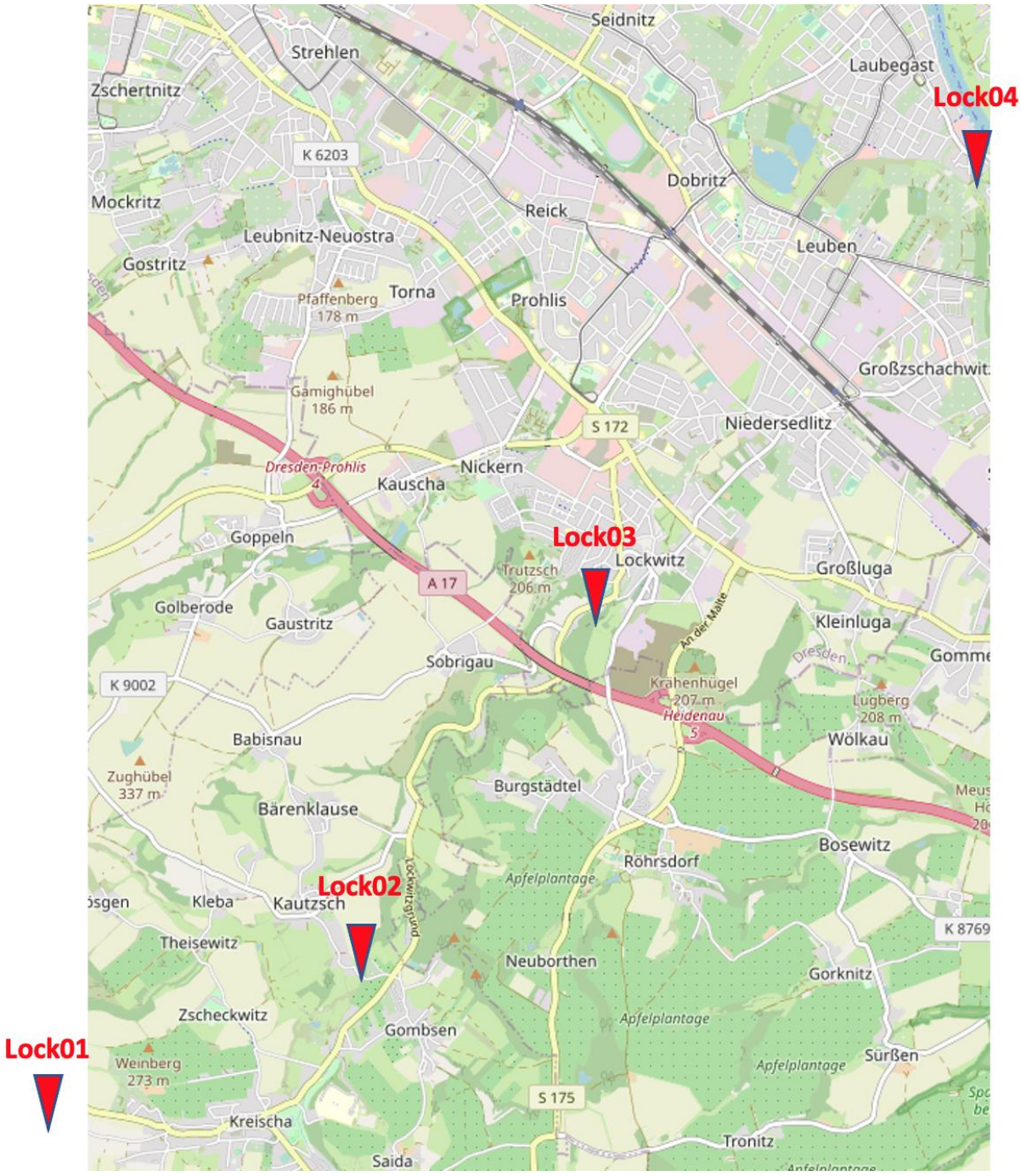

Figure 3: Map of the Lockwitzbach catchment with sampling site positions.

Figure 4 shows the locations of the samplers in the Geberbach catchment (area 11.9 km^2, river length 10.5 km). Its upstream part of approximately 5 km length is characterised by

agriculture. It is followed by the Kauscha flood protection reservoir at the Dresden city boundary. Downstream of the reservoir, the Geb1 baseline LVSPE sampler, but no event sampler was installed, as no runoff events are expected here. The downstream catchment is a largely urbanised with several surface-runoff/rainwater inlets. Here the baseline results from Geb1 will be a proxy for both Geb2 and Geb3.

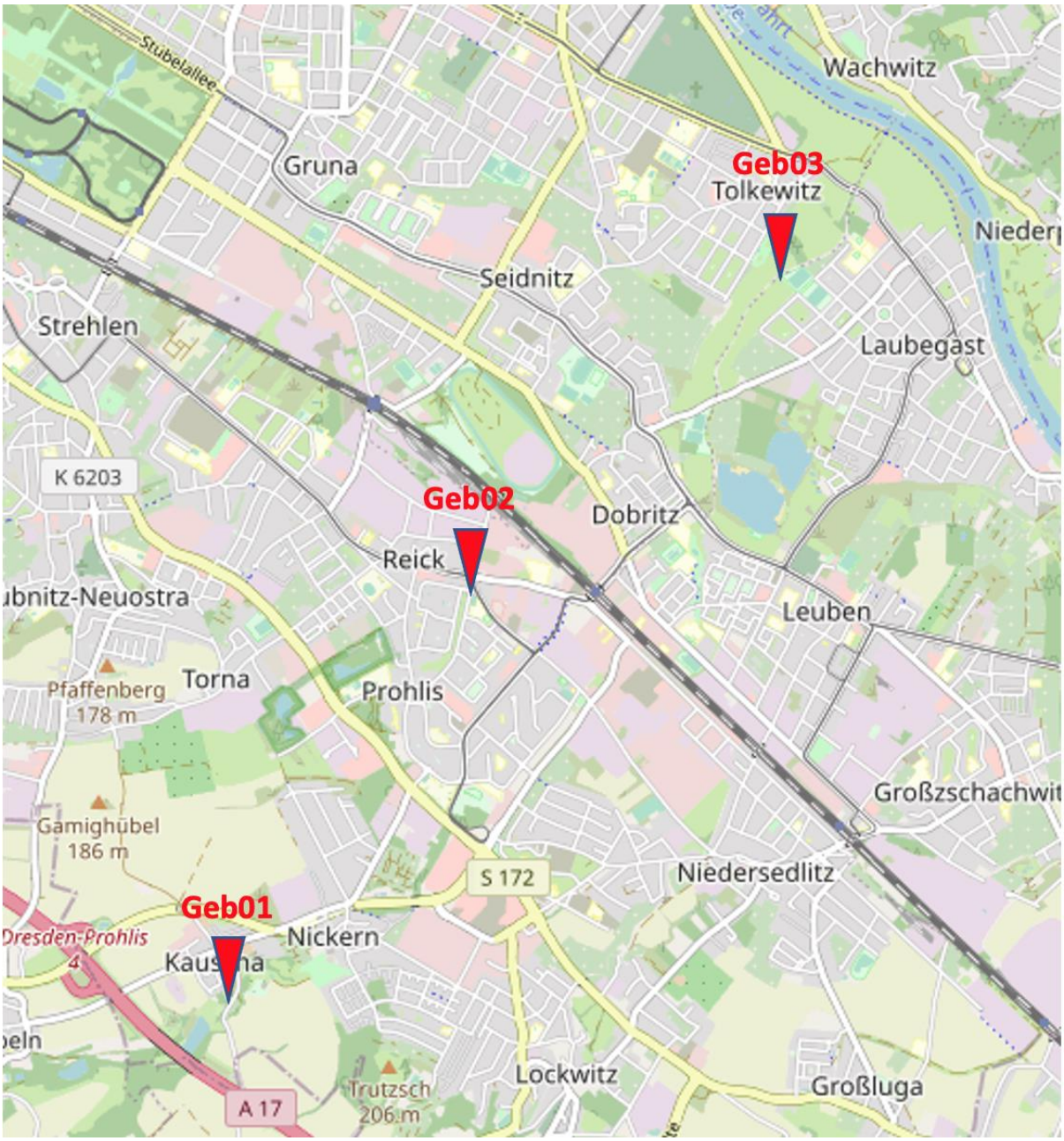

Figure 4: Map of the Geberbach catchment with sampling site positions.

Figure 5 shows the locations of the samplers in the Launzige catchment (area 19.7 km^2, river length 10.3 km). The whole catchment is dominated by agriculture. The village of Fremdiswalde located between Laun1 and Laun2 has no centralised WWTP, and on-site

observations saw pipes coming almost directly from the houses into the stream, indicating untreated wastewater inputs or effluents from small-scale on-site treatments. From June 2022 onwards upstream of the pond which is where Laun1 is situated was completely dry, therefore only 2 baseline samples were collected for this site.

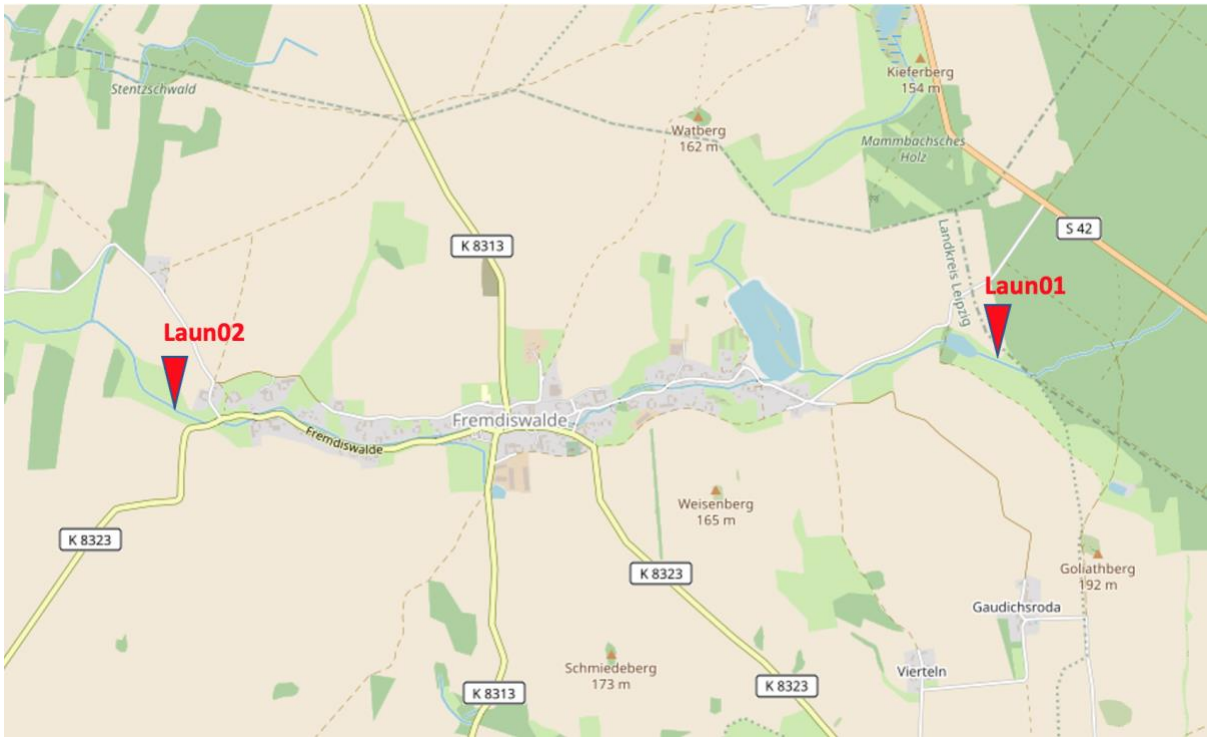

Figure 5: Map of the Launzige catchment with sampling site locations.

3.2 LVSPE Technical description

The devices were placed in a stable position close to the sampling sites. A long tube connected the device to the water body being sampled with a coarse filter at the entrance of the tube (1 mm) to prevent larger particles from entering the system or blocking the tube. A control unit within the device controlled the frequency and volume of samples taken and triggered the device to take samples accordingly. During sampling, water was sucked by vacuum through a prefilter to remove suspended particulate matter into a dosing vessel. This then released the exact volume into the pressure chamber where the sample was pushed through the cartridge. Samples flow through the cartridge from bottom to top to avoid preferential flow paths through the solid phase. An image of the device is shown in figure 6.

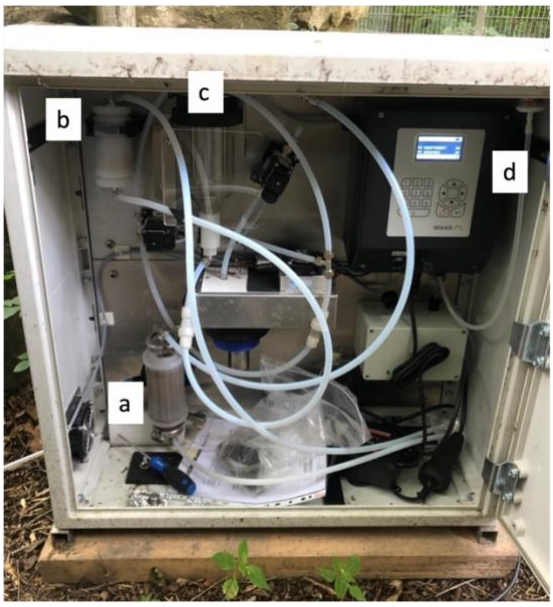

Figure 6:Image of the inside of an LVSPE device in the field where (a): prefilter, (b): SPE cartridge, (c): dosing unit, and (d): control unit.

The extraction cartridge is made of polyvinylidene fluoride, lower and upper screw caps secure the in- and outlet tubes to the cartridge. Inside the cartridge, the sorbent is held in place by a glass filter disk tightened with a silicone O-ring. The cartridges can be tailor made with different sorbents for the selection of different compounds, in this study Chromabond HR-X absorbent was used. A schematic view of the cartridge is shown in figure 7a.

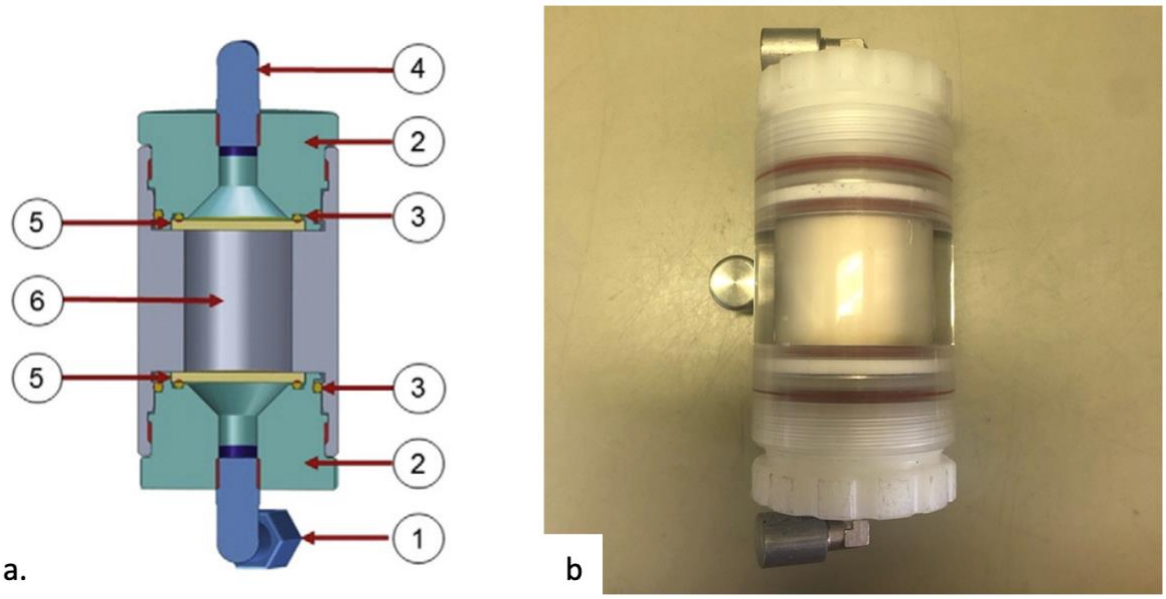

Figure 7a: a schematic of the extraction cartridge in the LVSPE device where (1): inlet fitting, (2): upper and lower screw caps, (3): O-rings, (4): outlet fitting, (5): glass filter disc, (6): body containing sorbent (Taken from Schulze et al., 2017). b: an image of a clear extraction cartridge showing the parts inside.

3.3 Materials and reagents

The LC-MS grade water was obtained from Fisher Scientific. LC-MS grade methanol, acetonitrile, ethyl acetate were obtained from Chromasolv®, Honeywell. Formic acid, eluent additive for LC-MS and ammonium fluoride, ≥99%, eluent additive for LC-MS were both obtained from Honeywell. 7 N Ammonia in methanol was obtained from Sigma-Aldrich. Formic acid, analysis grade 98-100% was obtained from Merck. The Chromabond HR-X sorbent was obtained from Macherey-Nagel. The syringe filter PTFE, pore size 0.2 µm was obtained from Whatman. The internal standard mix of 47 compounds was prepared by the WANA department at the UFZ using standards from various suppliers all with a minimum purity of 97%.

3.4 Solid phase extraction

3.4.1 LVSPE cartridge conditioning

All parts of the cartridge were cleaned with MeOH prior to being assembled. Cartridges are then assembled as described in section 3.2. The HR-X sorbent was cleaned at the UFZ by rinsing it for approximately 8 hours in LC-MS grade acetone in Soxhlet apparatus before being placed in the cartridges. Conditioning of the sorbent was done with a sequence of 200 mL ethyl acetate, 200 mL MeOH, and 100 mL LC-MS grade water. The flow through the cartridge was stopped before air bubbles could pass through to prevent the sorbent from drying. Caps were then put on the cartridges, and they were stored in the fridge at 4 °C for up to 2 days before use.

3.4.2 Sampling

Samples were taken using LVSPE devices which took samples automatically over a period of 5 months. The LVSPE cartridges had the capacity to take 20 L of water; therefore for 28-day baseline sampling they took 60 mL of water every 2 hours. A sensor monitoring water level height would trigger the devices to take event samples after rainfall. Event samplers took 500 mL every 5 minutes for a total of 3 hours 20 minutes. The total volume collected was recorded, because in some cases less than 20 L was collected due to the prefilters being blocked or the exact sample collection time varying slightly. After collection, the cartridges were stored in a cooler box for transport from the site to the lab and stored in the freezer.

Cartridges were then blown down and freeze dried to remove the remaining water. Table 3 shows a complete list of all samples taken.

Table 3: Tabling showing the collection dates of all samples taken.

Site	Lock 1	Lock 2	Lock 3	Lock 4	Geb 1	Geb 2	Geb 3	Laun 1	Laun 2
Baseline	12.05.22	12.05.22	12.05.22		12.05.22			11.05.22	11.05.22
	08.06.22	08.06.22	08.06.22		08.06.22			09.06.22	09.06.22
	06.07.22	01.08.22	06.07.22		06.07.22				07.07.22
	01.08.22	30.08.22	01.08.22		01.08.22				02.08.22
	30.08.22		30.08.22		30.08.22				30.08.22
Event		17.05.22	17.05.22	17.05.22		24.04.22	17.05.22		30.07.22
		28.06.22	28.06.22	28.06.22		17.05.22	04.06.22		19.08.22
		23.08.22	23.08.22	23.08.22		27.05.22	28.06.22		26.08.22
				27.08.22		30.05.22	07.06.22		
						04.06.22	09.07.22		
						06.07.22	23.08.22		
						09.07.22	26.08.22		
						23.08.22			
						31.08.22			

3.4.3 Elution + LCMS prep

Samples were eluted from the SPE cartridges with a sequence of 100 mL each of ethyl acetate, methanol, 1% formic acid in methanol by volume, and 2% ammonia in methanol by volume. Samples were then evaporated to a few mL and transferred to vials, then topped up with methanol to reach the volume which equates to an enrichment factor (EF) of 1000, according to the sample volume collected by the LVSPE. 2 mL vials with 200 µL glass inserts were filled with 100 µL of sample, 30 µL of LC-MS grade MeOH, 60 µL of LC-MS grade H_2O, and 10 µL of internal standard mix 1 µg mL^{-1} for LC-HRMS analysis. After the first round of measurements vials were also prepared at various diluted EFs between 50 and 0.5 to adjust measured concentrations above to levels within the calibration range in a second round of analysis.

3.5 Calibration

A method-matched calibration curve was created by filtering batches of 1 L of uncontaminated surface water from a pristine creek in the upper Harz mountains (Womsgraben) before adding a mixture of the target compounds at the dedicated calibration levels shown in table 4. A laboratory-scale SPE was then performed for each calibration point using a Promochrom SPE-03 device. Extracts were then eluted sequentially using 5 mL each of ethyl acetate and methanol, then 4 mL each of 1% formic acid in methanol by volume and

2% ammonia in methanol by volume, before being reduced under nitrogen to roughly 500 µL and passed through a 0.2 µm filter. Extracts were then reduced to dryness and sequentially topped up with MeOH to reach an EF of 1000. Calibration samples were then prepared for analysis by LC-HRMS with EF500 the same way as the samples.

Table 4: Calibration standard concentrations of individual analytes.

Calibration point	1	2	3	4	5	6	7	8	9	10	11
Concentration/ ng L-1	0.5	1	2	5	10	20	50	100	200	500	1000

3.6 Instrumental analysis

LC-HRMS measurements were made using a Thermo Ultimate 3000 LC system combined with a Thermo QExactive Plus with ESI source. All samples were run in both positive and negative mode (ESI+/ESI-) in separate runs. Reverse-phase liquid chromatography was performed using a Kinetex Biphenyl column (100 x 2.1 mm, 2.6 µm particle size, Phenomenex) and a water: methanol: acetonitrile gradient using 0.1% formic acid (nominal aqueous pH 2.6) in ESI+ mode and 1 mM ammonium fluoride in ESI- mode. The gradient program was the same for positive and negative mode and is shown in table 5. The flow rate was 300 µL min^{-1}, the column oven temperature 40°C and the runtime per sample was 29 minutes. The injection volume was 5 µL. The QExactive ran full MS scans from m/z 80-1200 at a nominal resolving power of 70,000 (referenced to m/z 200), combined with 6 data independent acquisition MS^2 scans in different m/z windows and two stepped collision energies (m/z 80-182, CE 45+65 a.u.; m/z 178-282, CE 35+55; m/z 278-382, CE 30+55; m/z 378-482, CE 30+50; m/z 478-682, CE 25+50; m/z 678-1200, CE 20+45) at a resolving power of 35,000. The spray voltage was 3800 V in ESI+ and 3500 V in ESI- mode, the sheath gas flow rate 45 a.u., the auxiliary gas flow rate 1 a.u., and the auxiliary gas temperature 300°C in both modes.

Table 5: The solvent gradient program used by the Thermo Ultimate 3000 LC system.

Retention/ min	Flow/ mL min-1	% H20	% MeOH	% MeCN
0.000	0.300	97.0	3.0	0.0
2.000	0.300	97.0	3.0	0.0
16.000	0.300	97.0	3.0	0.0
20.000	0.300	3.0	97.0	0.0
24.000	0.300	3.0	0.0	97.0
24.250	0.400	3.0	0.0	97.0
28.800	0.350	97.0	3.0	0.0

3.7 Data analysis

The obtained Thermo.raw files were converted to an mzML format using ProteoWizard (v. 3.0.18265) (Chambers et al., 2012) and processed using the software MZmine 2.38 (Pluskal et al., 2010) for peak picking, deconvolution, alignment, gap-filling, and peak annotation as detailed in Beckers et al. (2020). The UFZ in-house R package MZquant (Schulze et al., 2021) was used for the semi-automated quantification of compound concentrations. A generalized additive model was used for calibration, which was trimmed to the detection range with at least four data points (calibration levels) included. Some compounds were checked manually, using the vendor software TraceFinder (version 4.1, Thermo Scientific). These were either quality controls (QCs) or compounds, which could not be reliably annotated automatically based on previous experience. In total 735 compounds were analysed.

For the calculation of toxic units (TUs) (Explained in section 2.1.2) EC_{50} or LC_{50} data were selected in the order: (1) Experimental data retrieved from US EPA ECOTOX Knowledgebase (US EPA, 2021) and (2) predicted data using the ECOSAR type baseline model for the BQE fish, daphnia, and green algae in ChemProp 6.7.1 (UFZ Department of Ecological Chemistry, 2019). For (1) a statistical value was calculated based on this data which represented the 5^{th} percentile of all effect concentrations available per biological endpoint. This data was then filtered and transformed as detailed in Schulze et al., (2021).

4. Results and Discussion

4.1 Overview

Of the 793 target chemicals in the screening 164 compounds were not found in any baseline or event samples across all catchments. 279 compounds were not detected in the Lockwitzbach catchment, 261 in the Geberbach catchment, and 297 in the Launzige

catchment. 58 compounds could not be analysed as it was not possible to obtain a good calibration curve, these were mostly phthalates and some other polymer additives and surfactants. For these compounds a high level of background contamination was present, or a low recovery was seen, or there was interference from other compounds in the LC-HRMS data. Out of the 671 compounds with at least one detection there were 468 with toxicity data allowing for the calculation of TUs.

4.2 Comparison of baseline and event samples

4.2.1 Lockwitzbach indicates inputs from a range of urban sources, rain events have relatively little impact on concentration of the majority of compounds.

The polymer additive benzothiazole had the highest concentration with 1.5 µg L^{-1} (Figure 8). Benzothiazole and its derivatives are used mainly as vulcanisation accelerators in rubber production, therefore tyre production (Kloepfer et al., 2004; Wagner et al., 2018). 3-cyclohexyl-1,1-dimethylurea is also used as a vulcanisation accelerator in rubber and an additive in hydraulic fluids and lubricating oils and was also found at a high concentration with 780 ng L^{-1}. The benzothiazole derivative 2-benzothiazolesulfonic acid was also detected at 590 ng L^{-1}. These compounds indicate some input from road runoff. The sweetener sucralose had the second highest concentration with 1.3 µg L^{-1}. As previously discussed in section 2.1.1 sucralose often is detected in the µg L^{-1} range, and the downstream portion of the Lockwitzbach catchment is heavily urbanised, which can explain the presence of sucralose. 1H-benzotriazole was the third highest concentration detected with 790 ng L^{-1}, this compound is a corrosion inhibitor often used in dishwasher detergents. The high concentrations of this and sucralose as well as some other pharmaceuticals indicates some influence from domestic wastewater.

Figure 9 shows the same for the event samples within the Lockwitzbach catchment. The highest concentration in the event samples was that of 2-benzothiazolesulfonic acid at 3.2 µg L^{-1}. Benzothiazole was also detected at 2.0 µg L^{-1}. It is expected that TRWPs and TRWP related pollutants are transported via surface runoff to surface waters, which may explain why concentrations of benzothiazoles are notably higher in the event samples. The solvent 2-(2-(2-methoxyethoxy)ethoxy)ethanol was detected at a concentration of 2.3 µg L^{-1}. Data of

common environmental concentrations of this compound in surface waters were rare but this compound has a variety of uses as an additive in paints, hydraulic and brake fluids, and plasticisers. Its presence at high concentrations along with other traffic-related compounds also suggest inputs from road runoff. Sucralose and 1H-benzotriazole were also detected at 1.7 and 1.2 µg L^{-1} respectively, along with other personal care and household and food and beverage compounds, indicating domestic wastewater inputs. The natural compounds rutin and hyperoside were also detected with concentrations of 1.3 and 1.1 µg L^{-1} respectively, both of which are known to be detected in surface waters in the ng to µg L^{-1} range (Nanusha et al., 2020, 2021). The presence of the two natural compounds in such high concentrations could indicate that the plant species which produce them are abundant in this catchment and these are leached from plant material.

Figure 10 shows the compound classes of the 100 compounds detected at the highest concentrations in the baseline and event samples in the Lockwitzbach catchment. In both the baseline and event samples the largest class in the top 100 compounds is pharmaceuticals making up 29% and 23% respectively, followed by polymer additives making up 18% and 22% respectively. During events, the percentage of pesticides decreases from 15% to 9% which is unsurprising in this mostly urban catchment. The percentage of personal care and household compounds increases slightly from 10% to 11% which is an indicator that the events in this catchment have some inputs of untreated domestic wastewater, likely coming from the CSOs. The inputs from treated and untreated wastewater are discussed in section 4.2.4. The fractions of PCPs and household compounds increase whilst pharmaceuticals decrease despite them both coming from domestic wastewater, likely because personal care and household products are generally more well removed than pharmaceuticals. When there are inputs from untreated wastewater absolute concentrations of pharmaceuticals and PCPs are both affected, but for PCPs which are usually well removed the concentration is increasing by more and therefore taking up a larger fraction of the top 100 detected compounds.

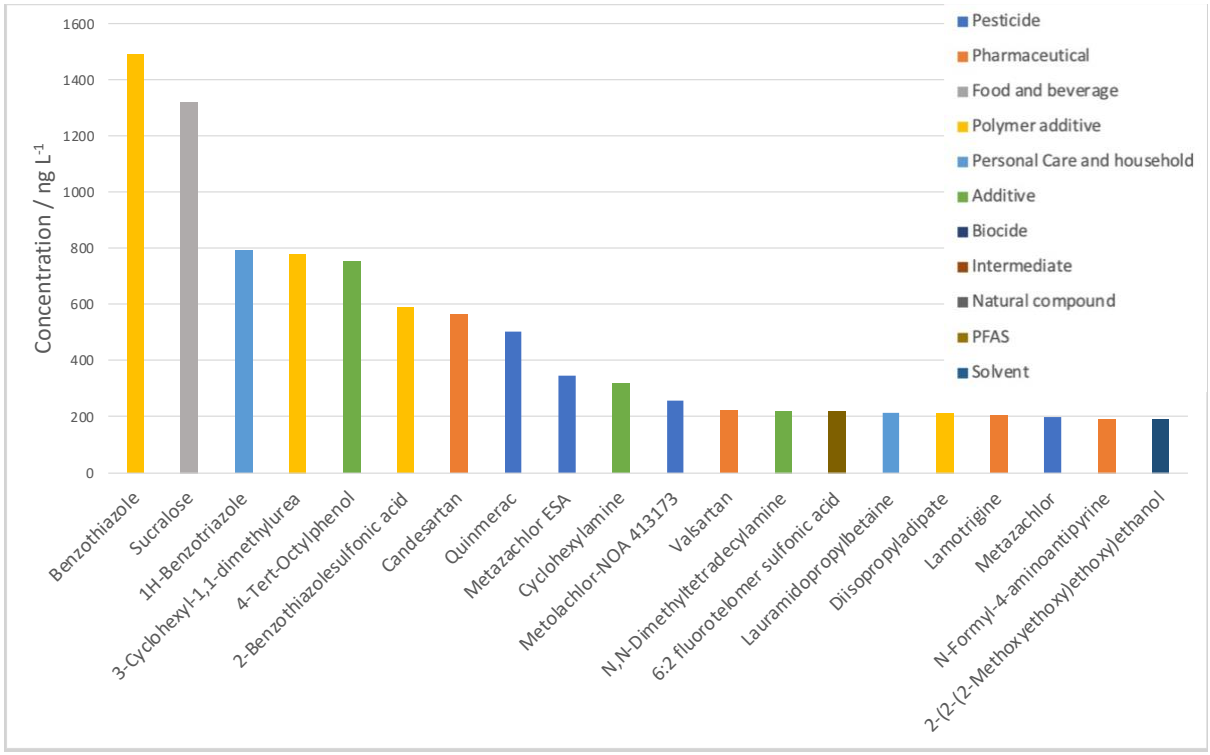

Figure 8: 20 Highest mean concentrations found across all Lockwitzbach baseline samples.

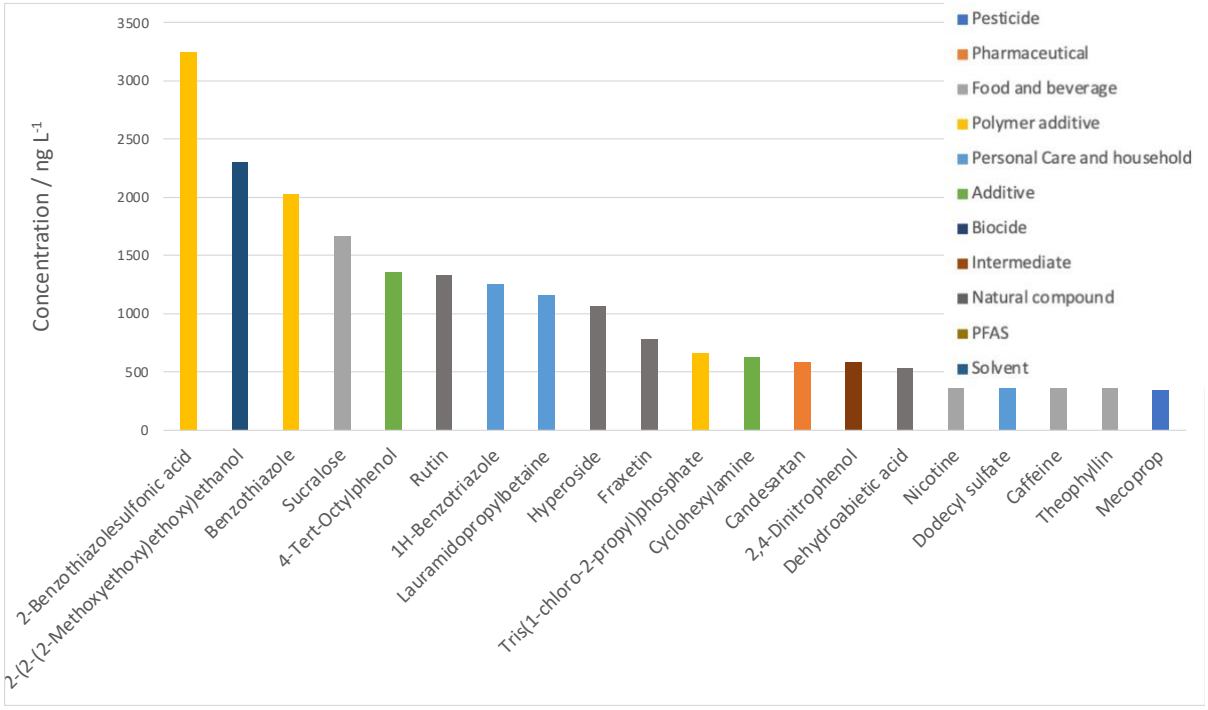

Figure 9: 20 Highest mean concentrations found across all Lockwitzbach event samples.

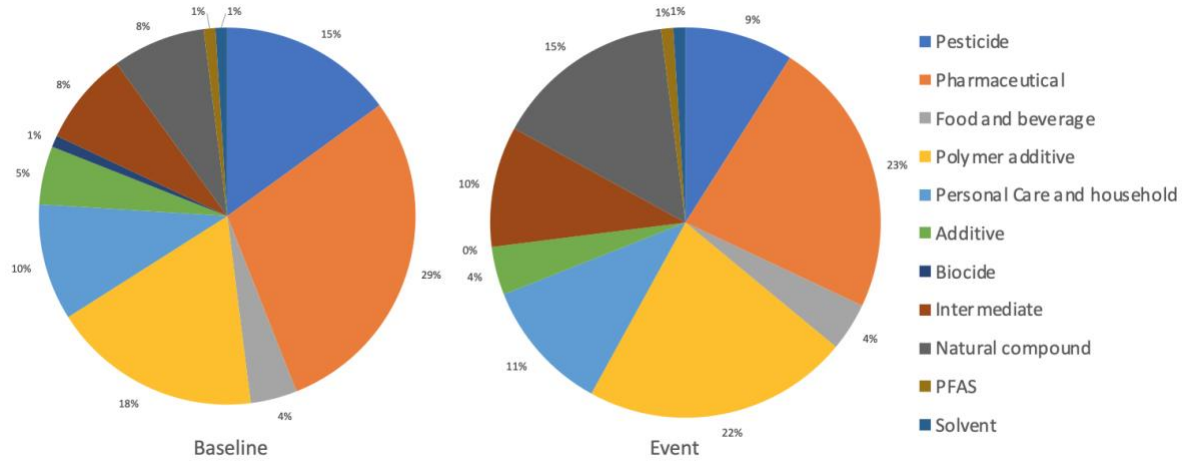

Figure 10: Pie charts showing the compound classes as a percentage of the 100 compounds with the highest concentration detected across Lockwitzbach baseline and event samples respectively.

Figure 11 shows the difference in average concentration and variation of compounds between baseline and event samples for pharmaceuticals (stemming likely from point sources) and pesticides (stemming likely from diffuse sources). Here only the data from sites Lock 3 and 4 were used as it was assumed concentrations do not vary significantly upstream of the urbanised area, in which the majority of the CSOs are located. For both classes the average concentrations do not appear to be higher in either the baseline or event samples. For the pesticides, whilst there are three compounds with a comparably high variation in the event samples (propamocarb, pirimicarb, and dimethoate), the variability does not differ between the baseline and event samples. This could potentially be because this portion of the catchment is heavily urbanised and so increased pesticide inputs into the stream are diluted again downstream by surface runoff and sewer overflows. For the pharmaceuticals there is a very slight increase in variation in the event samples, showing that rain events have only a small effect here. This could be because the large rehabilitation hospital near Kreischa is likely the main constant source of pharmaceuticals in this catchment dominating the baseline concentrations and therefore the input from CSOs further downstream during events does not affect the average concentrations very much. It could also be the case that the rain events were strong enough to trigger sampling but not necessarily strong enough to cause all CSOs to overflow. Scatter graphs for other compound classes were created and can be found in appendix A. Roughly the same patterns are found for each compound class, that is, roughly similar concentrations between baseline and event samples but higher variation in the event samples.

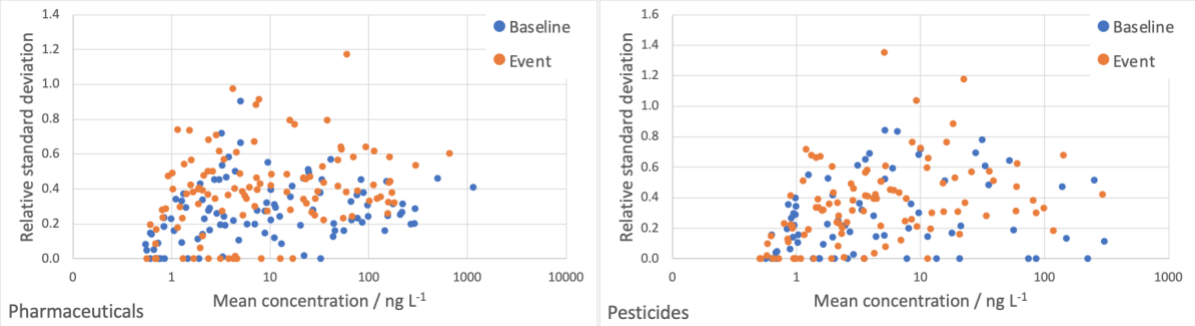

Figure 11: Scatter graphs showing the mean concentration vs the relative standard deviation for both pharmaceuticals and pesticides and highlighting the difference between baseline and events in the Lockwitzbach catchment.

4.2.2 Road run-off compounds dominate in the Geberbach, rain events cause an increase in concentration for all compound classes.

Figure 12 shows the 20 compounds which had the highest concentrations of all baseline samples from the Geberbach catchment. The polymer additive benzothiazole was detected at the highest concentration with a concentration of 2.2 µg L^{-1}. This was followed by 2-benzothiazolesulfonic acid and 3-cyclohexyl-1,1-dimethylurea with 840 and 710 ng L^{-1} indicating there is likely also a significant input from road-runoff, which is consistent with the fact it is a highly urbanised catchment. The additives 4-tert-octylphenol and cyclohexylamine were detected at 580 and 520 ng L^{-1} respectively. 4-tert-octylphenol is an endocrine disruptor and therefore has limited use in the EU, however it is still used in coatings, sealants, and dyes. It has been shown that rainfall is an important source of 4-tert-octylphenol to surface water and has been detected in many rivers globally (Zhao et al., 2021; Salgueiro-González et al., 2019; Saha et al., 2022; Calderón-Moreno et al., 2019). Cyclohexylamine is commonly used as a corrosion inhibitor, however it is also a metabolite of the sweetener cyclamate (Renwick et al., 2004). Cyclamate has been detected in WWTP influents in high concentrations and cyclohexylamine has been detected in WWTP effluents in concentrations ranging from some ng L^{-1} up to 70 µg L^{-1} (Scheurer et al., 2009; Finckh et al., 2022).

In the event samples of the Geberbach (figure 13) the highest concentration is that of 2-benzothiazolesulfonic acid with 9.1 µg L^{-1}. The high concentrations of this and other benzothiazole compounds such as benzothiazole, 2-hydroxybenzothiazole, and 2-(methylthio)benzothiazole, indicate this catchment has a lot of road-runoff inputs, however the concentrations are noticeably higher in the event samples, highlighting that storm related runoff is a significant pathway for these pollutants. The second and third highest compounds

detected were Lauramidopropylbetaine and cyclohexylamine with 3.7 and 3.4 µg L^{-1} respectively. Lauramidopropylbetaine is a surfactant commonly used in shower gels and other PCPs. The presence of these two compounds suggests there is also some influence from domestic wastewater, which is also consistent with the fact that Geberbach is largely an urban catchment.

Figure 14 shows the compound classes of the 100 compounds detected at the highest concentrations in the baseline and event samples in the Geberbach catchment. In this catchment there is a large difference in pollutant mixture composition between baseline and event samples. Polymer additives dominate this catchment both in baseline and event samples, however under event conditions the proportion of polymer additives being detected at the highest concentrations increases from 22% to 33%. This supports the statement that this catchment is heavily influenced by traffic related pollutants and surface runoff, which is to be expected in this heavily urbanised catchment. There is also an increase in personal care and household products from 11% to 14%, indicating there is also some input from domestic wastewater. The percentage of pesticides being detected in the highest concentrations decreases from 18% to 6% which is unsurprising given the lack of agriculture in this area.

Figure 15 shows the average concentration and variation for pharmaceutical and pesticide compounds in the Geberbach catchment and highlights the difference between baseline and event samples. For the pharmaceuticals, the event samples exhibit higher concentrations which suggests wastewater inputs likely coming from CSOs. The concentrations also exhibit a larger variation in the event samples as compared to the baseline samples. For the pesticides, there are some high concentrations in baseline samples, but overall higher concentrations are exhibited in the event samples. In the event samples the pesticides show much more variation than the baseline samples, indicating that despite being a very urbanised area there is some pesticide input in this catchment, and its input to the Geberbach is heavily influenced by rain events. This could result from urban pesticide usage or from gardens in the less densely populated areas. Scatter graphs for other compound classes were created and can be found in appendix B. Roughly the same patterns are found for each compound class, that is a clear increase in concentration and variation in the event samples compared to the baseline samples.

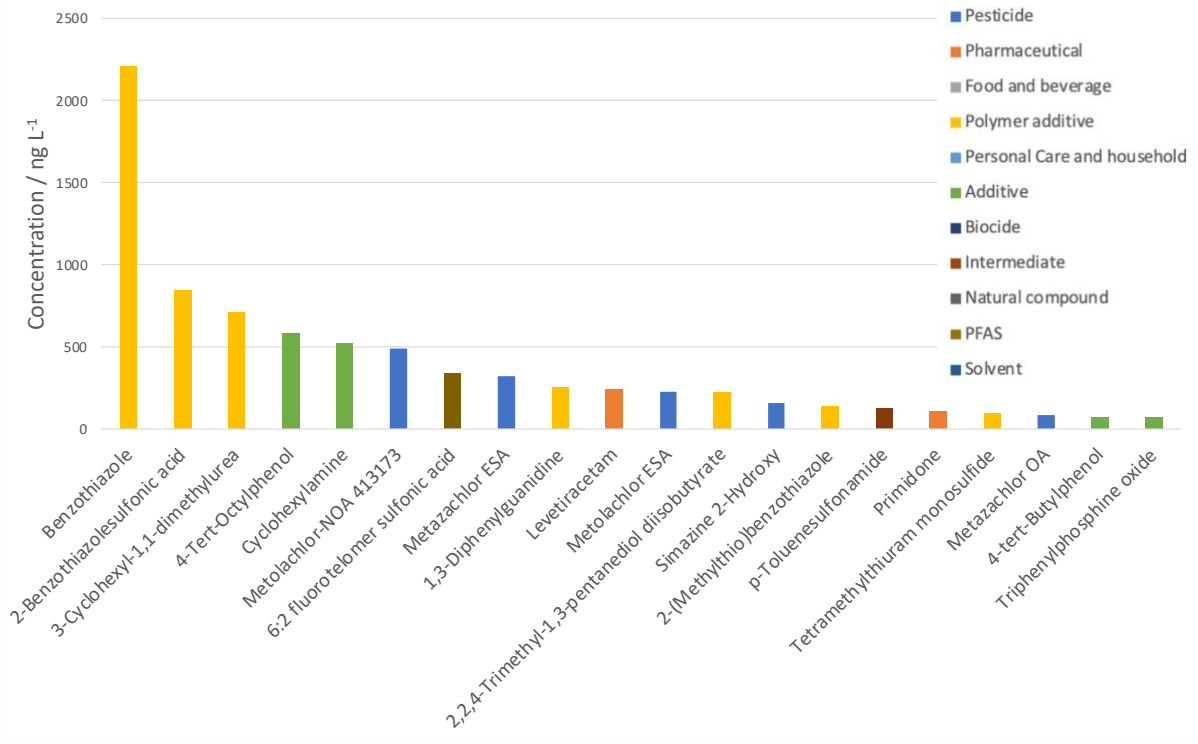

Figure 12: 20 Highest mean concentrations found across all Geberbach baseline samples.

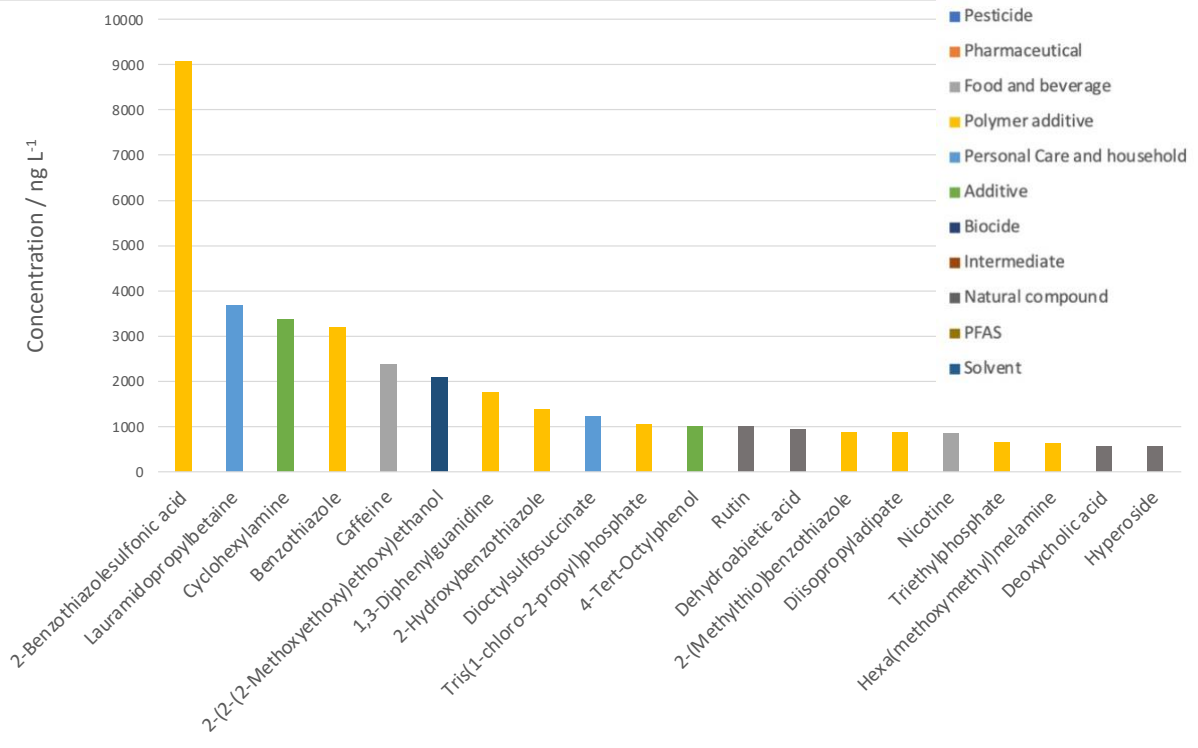

Figure 13: 20 Highest mean concentrations found across all Geberbach event samples.

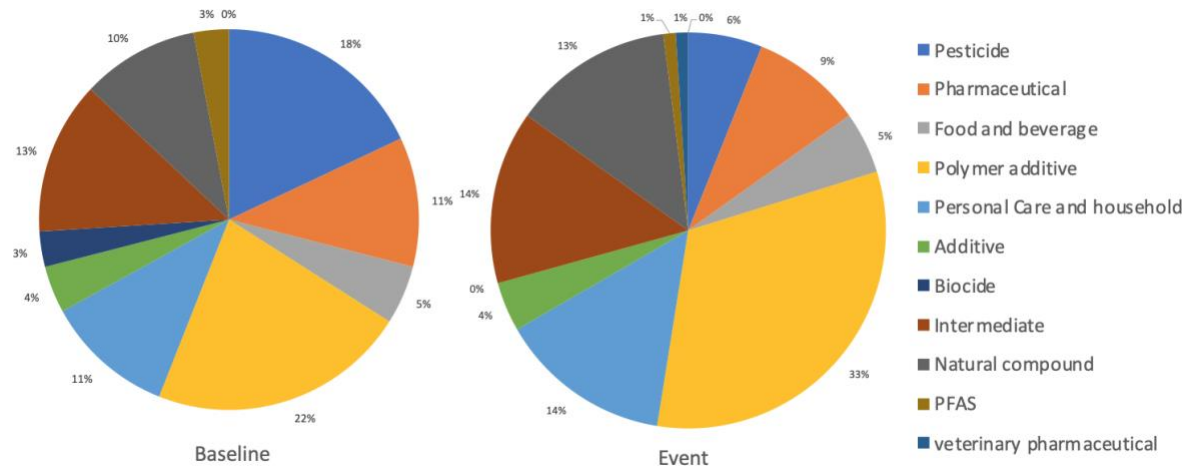

Figure 14: Pie charts showing the compound classes as a percentage of the 100 compounds with the highest concentration detected across Geberbach baseline and event samples respectively.

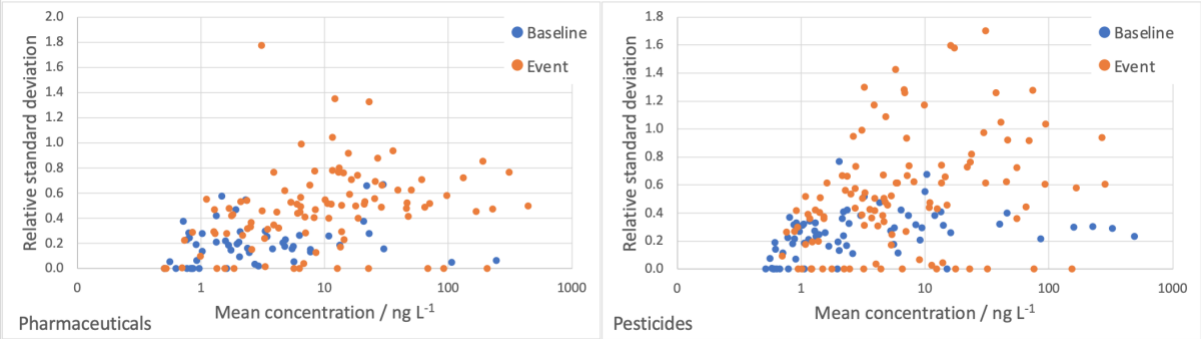

Figure 15: Scatter graphs showing the mean concentration vs the relative standard deviation for both pharmaceuticals and pesticides and highlighting the difference between baseline and event in the Geberbach catchment.

4.2.3 Rural Launzige shows highest inputs of pesticides, rain events have little impact on concentrations for the majority of compound classes.

Figure 16 shows the highest concentrations detected were those of pesticides, with the two transformation products of metazachlor being detected at the highest concentrations of 2.9 and 2.7 µg L^{-1} for metazachlor ESA and OA respectively. Metazachlor has a short half-life of 5-30 days in soil and transforms to its ESA and OA derivatives which are 100% deprotonated at the majority of soil pH values, leading to high mobility and frequent high concentrations in surface waters (Hvězdová et al., 2018). This catchment is relatively rural and influenced from agriculture, which could explain more pesticides appearing at the highest concentrations detected. Three metabolites of the pesticide metolachlor were found at some of the highest concentrations in this catchment during baseline conditions, with 1.9 µg L^{-1}, 1.1 µg L^{-1}, and 970 ng L^{-1} for metolachlor NOA 413173, metolachlor ESA, and metolachlor CGA 368208, respectively. Metolachlor and its metabolites are known to be detected in surface waters in Germany and Europe (Reemtsma et al., 2013; Huntscha et al., 2008). The pharmaceutical

compound N-Acetyl-4-aminoantipyrine was the fourth highest concentration detected with 2.1 µg L^{-1}. It is a metabolite of the analgesic Metamizole and is not uncommon in surface waters (Wiegel et al., 2004; Wu et al., 2023). Along with a high detection of sucralose at 2.5 µg L^{-1}, this indicates some inputs of domestic wastewater which is consistent with the on-site observations of pipes appearing to come directly from houses.

Figure 17 shows that in the event samples the highest concentration detected was that of the personal care product lauramidopropylbetaine at 5.3 µg L^{-1}. The Launzige catchment did however only have three events during the sampling period, with a standard deviation of 5.6. Other compounds which have previously been discussed and detected in the other catchments at high concentrations were also detected here, such as the polymer additives 2-benzothiazolesulfonic acid and benzothiazole, and the pesticide transformation product metazachlor ESA at 2.7, 2.2 and 2.1 µg L^{-1}, respectively. Despite low levels of traffic and not many roads in this catchment, benzothiazolesulfonic acid appears to be a fairly ubiquitous traffic related micropollutant appearing in relatively high concentrations here and across all three catchments.

Figure 18 shows the change in compound class composition in the top 100 detected compounds between baseline and event conditions in the Launzige catchment. Here pharmaceuticals make up the largest proportion with 29% and 21% in the baseline and event samples, respectively. A large proportion of pharmaceuticals indicates inputs of domestic wastewater, which is consistent with the on-site observations of pipes coming from housing into the stream directly. Pesticides also make up a large proportion in this catchment, with 22% and 19% in the baseline and event samples, respectively. These are the largest proportions of pesticides found in the three catchments which is also consistent with the fact that at the Launzige a large part of the catchment is used for agriculture. The proportion of natural compounds increases under event conditions, going from 7 to 15%, as does the proportion of personal care and household compounds, going from 8 to 12%.

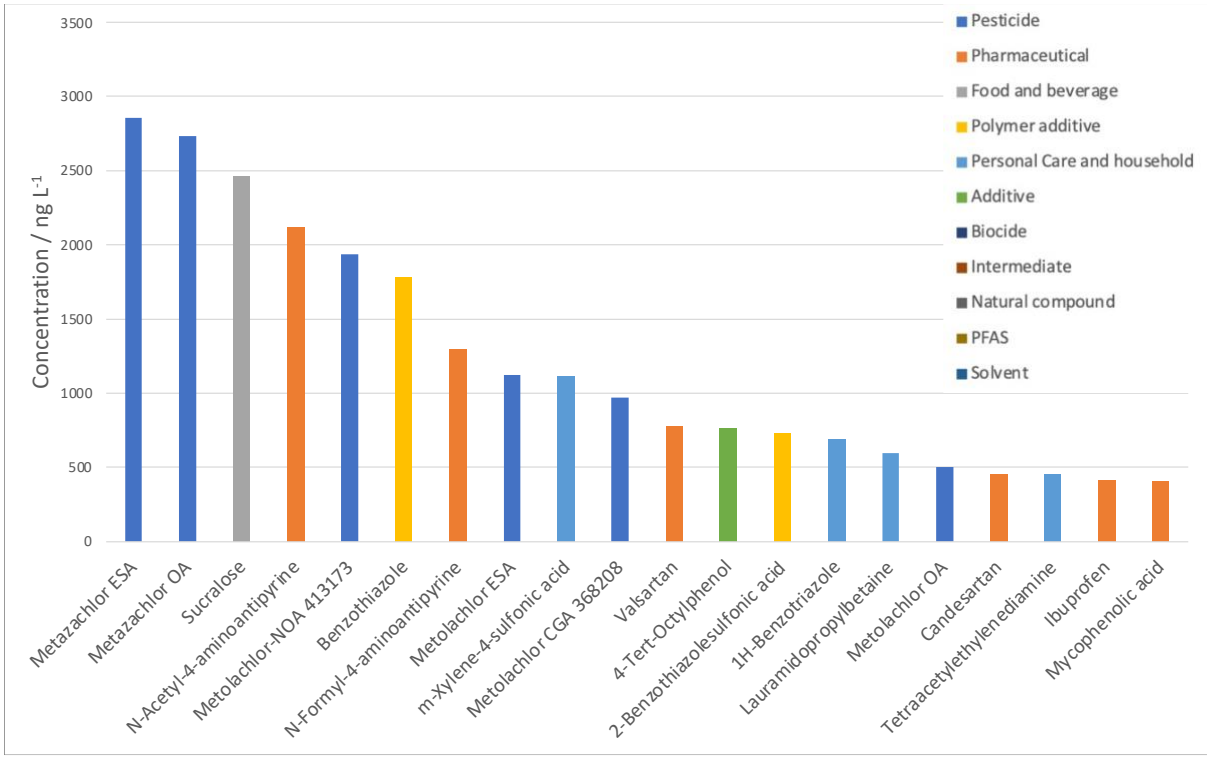

Figure 16: 20 Highest mean concentrations found across all Launzige baseline samples.

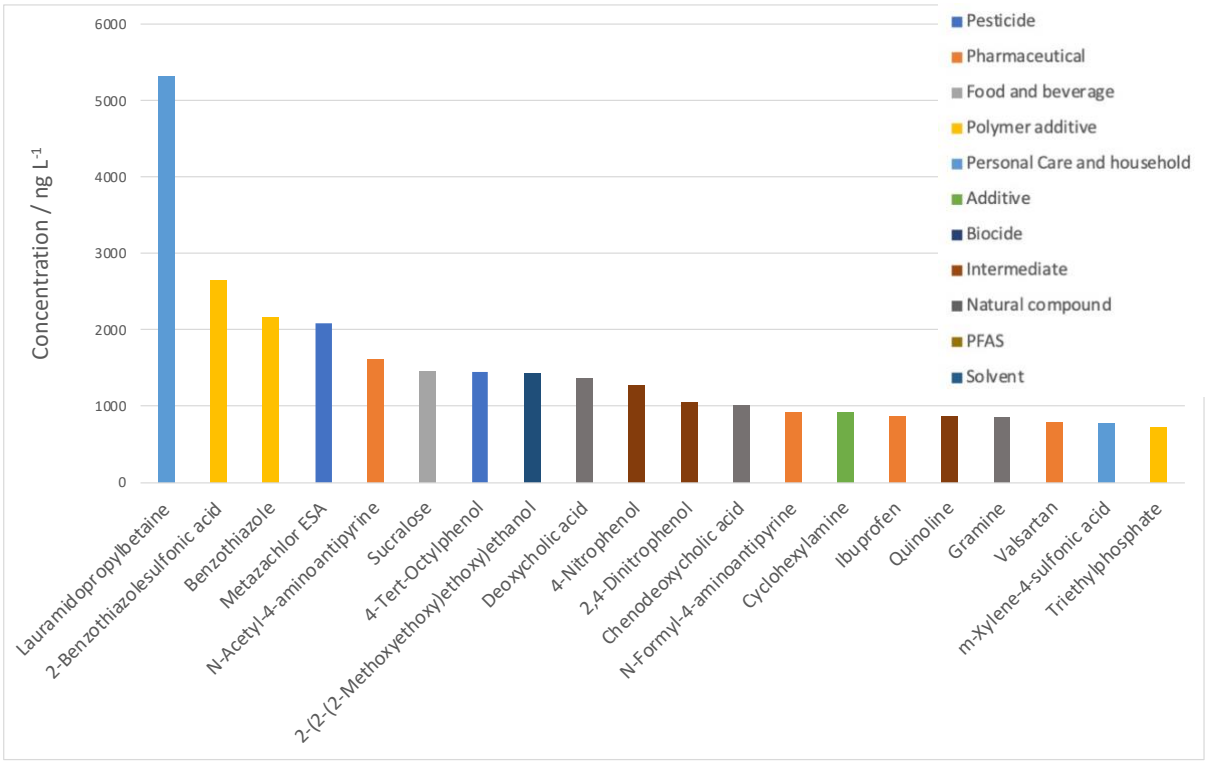

Figure 17: 20 Highest mean concentrations found across all Launzige event samples.

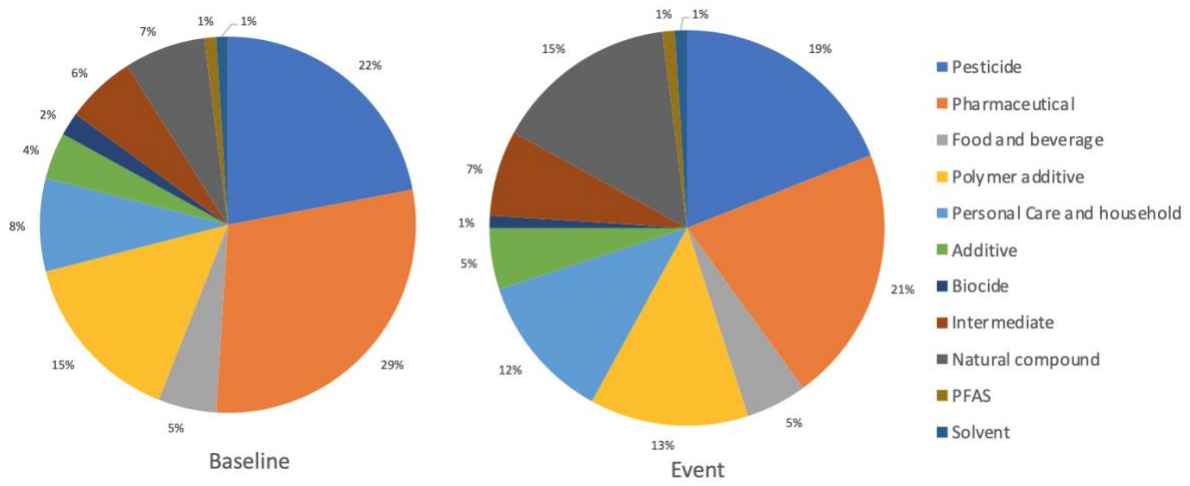

Figure 18: Pie charts showing the compound classes as a percentage of the 100 compounds with the highest concentration detected across Launzige baseline and event samples respectively.

Figure 19 shows the difference in mean concentrations and relative standard deviations under baseline and event conditions for both pharmaceuticals and pesticides. For the pharmaceuticals it seems that there is no difference in mean concentration or variation between the events and baseline samples. It could be that any extra pharmaceuticals entering the Launzige in event conditions are being cancelled out by dilution effects leading to roughly constant concentrations over the time span of this sampling campaign. Given that this area is known to have a lot of agricultural land use it could be expected that mean concentrations and variations for pesticides would be higher in events than in baseline conditions, however the concentrations seem to be slightly higher in the baseline and exhibit a higher variation (figure 15). Higher concentrations in the baseline could mean that any extra inputs from agricultural runoff are diluted from the increased water volume to lower concentrations than normally present. It could also be due to the events happening in July and August, which is outside of the usual pesticide application time in spring. Furthermore, it is worth considering that this catchment only had three rain events across the whole sampling period and therefore it is hard to comment on the accuracy of these results. Scatter graphs for other compound classes were created and can be found in appendix C. Roughly the same patterns are found for each compound class, that is roughly similar concentrations between baseline and event samples but a slightly higher variation in the baseline samples.

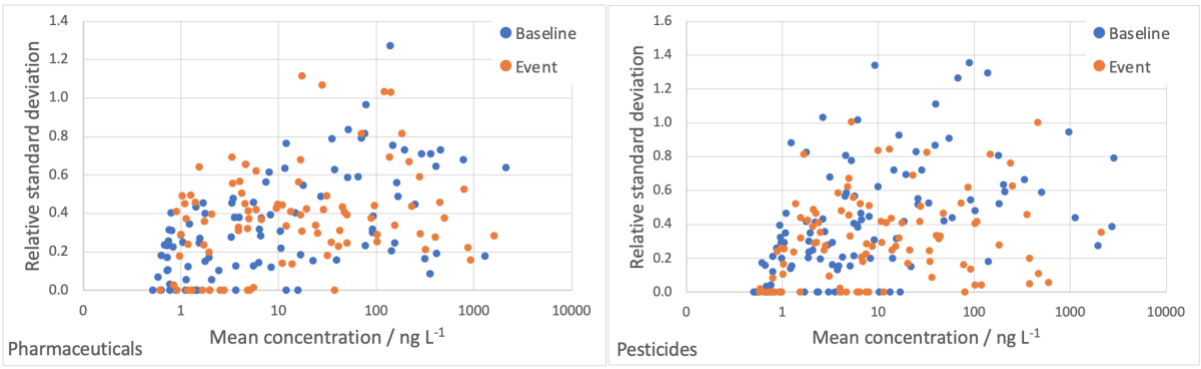

Figure 19: Scatter graphs showing the mean concentration vs the relative standard deviation for both pharmaceuticals and pesticides and highlighting the difference between baseline and event in the Launzige catchment.

4.2.4 Geberbach shows highest relative inputs of untreated wastewater, rain events increase untreated wastewater input across all catchments.

The ratio of sucralose to caffeine can be used as a qualitative indicator for the dominance of treated or untreated wastewater in a sample. Sucralose passes through WWTPs without being removed whereas caffeine is typically well removed (Oppenheimer et al., 2011; Buerge et al., 2006). A high concentration of sucralose indicates wastewater inputs, treated or untreated, whereas a low concentration of sucralose indicates there is no wastewater input. A high concentration of caffeine indicates inputs of untreated wastewater, whereas a low concentration indicates the input of either treated or no wastewater. The ratio of sucralose to caffeine can therefore be used to compare the untreated wastewater input between the three catchments.

Table 6 shows the concentrations of sucralose and caffeine and their ratios for both baseline and event conditions in each catchment. For each of the three catchments there is a clear increase in the relative amount of untreated wastewater in the event samples compared to baseline samples, which could be due to the presence of CSOs.

The Lockwitzbach shows a slight mean increase in wastewater input during rain events as indicted by an increase in sucralose concentrations from 1.3 to 1.7 µg L^{-1} , whilst the untreated wastewater fraction increases about 3-fold during rain events. This catchment shows the highest influence of wastewater during rain events; however, it also shows the lowest influence of untreated wastewater of all catchments during both baseline and event conditions.

The Geberbach shows a 10-fold increase of wastewater inputs during rain events whilst also having the highest input of untreated wastewater during rain event conditions as indicated by a 20-fold decrease of the sucralose/caffeine concentration ratio. Under baseline conditions however, the Geberbach shows the lowest mean concentrations of both sucralose and caffeine with 23 and 10 ng L^{-1}, respectively. This suggests a large influence from the CSOs in this catchment.

The Launzige actually shows a dilution effect of the wastewater under event conditions as the mean concentration of sucralose decreases from 2.5 to 1.5 µg L^{-1}. The untreated wastewater fraction however increases, as indicated by a decrease of the concentration ratio sucralose/caffeine from 7 to 2.

Table 6: Concentrations and concentration ratios of sucralose to caffeine in each catchment in both baseline and event samples, whereby high values indicate relatively treated wastewater and low values indicate relatively untreated wastewater.

		Lock BL	Lock EV	Geb BL	Geb EV	Laun BL	Laun EV
Sucralose ng/L	mean	**1320**	**1661**	**23**	**283**	**2464**	**1464**
	highest	3150	2641	28	791	3488	1887
	lowest	94	215	0	37	1405	943
Caffeine ng/L	mean	**101**	**371**	**10**	**2389**	**338**	**714**
	highest	181	1033	23	4699	436	1076
	lowest	n.d.	137	5	n.d.	261	436
Ratio	mean	13.1	4.5	2.3	0.1	7.3	2.1
	highest	17.4	2.6	1.2	0.2	8.0	1.8
	lowest	n.a.	1.6	0.0	n.a.	5.4	2.2

4.3 Environmental risk assessment

4.3.1 Rain events cause an exceedance of risk thresholds for every biological endpoint in all catchments.

Table 7 shows the ΣTU of each biological endpoint per catchment for both baseline and events. For all catchments and biological endpoints, the ΣTU increases during rain events which coincides with a general increase in concentrations during events. Despite the Launzige appearing to have overall similar or higher concentrations in the baseline samples, the compounds which are the main toxicity drivers of the ΣTU do indeed increase in concentration during events.

Using the ΣTU chronic risk thresholds suggested by Malaj et al. (2014) of 0.02 for algae, 0.001 for crustaceans, and 0.01 for fish, it was found that at the majority of sites the measured chemicals pose a chronic risk for the respective biological endpoints, and thresholds are exceeded for every biological endpoint in every catchment during rain events.

Table 7: The mean, highest, and lowest value for the sum TU of each biological end point for baseline and event samples in all three catchments.

Sum TU Algae						
	Lock BL	Lock EV	Geb BL	Geb EV	Laun BL	Laun EV
Mean	0.0090	0.0266	0.0109	0.0564	0.0323	0.0487
Highest	0.0280	0.0460	0.0198	0.1052	0.0596	0.0519
Lowest	0.0025	0.0108	0.0082	0.0089	0.0023	0.0427

Sum TU Crustaceans						
	Lock BL	Lock EV	Geb BL	Geb EV	Laun BL	Laun EV
Mean	0.0152	0.0311	0.0153	0.0651	0.0328	0.0668
Highest	0.0327	0.0489	0.0200	0.1725	0.0598	0.0784
Lowest	0.0028	0.0132	0.0102	0.0088	0.0025	0.0561

Sum TU Fish						
	Lock BL	Lock EV	Geb BL	Geb EV	Laun BL	Laun EV
Mean	0.0029	0.0134	0.0086	0.0425	0.0080	0.0194
Highest	0.0096	0.0275	0.0177	0.0791	0.0224	0.0248
Lowest	0.0009	0.0068	0.0060	0.0073	0.0015	0.0144

ΣTUs for algae

For the Lockwitzbach catchment during baseline conditions the chronic risk threshold of 0.02 for algae is not exceeded based on the mean, however one sample did exceed this threshold. During event conditions the risk threshold is exceeded with a mean ΣTU_{Algae} of 0.0266, however three samples were under the threshold.

For the Geberbach catchment the risk is not exceeded during baseline conditions in any sample, although the highest sample detected close to the threshold with 0.0198. During rain events the risk is exceeded in all but one of the samples, with a mean ΣTU_{Algae} of 0.0564 and some values going up to an order of magnitude above the threshold.

In the Launzige the risk is exceeded both during baseline and event conditions with ΣTUs_{Algae} of 0.032 and 0.049 respectively. Of the baseline samples there are two which do not exceed the risk threshold. In every event sample the threshold is exceeded by at least a factor of 2.

ΣTUs for crustaceans

For all catchments, the chronic risk threshold of 0.001 for crustaceans is exceeded in all samples across both baseline and event conditions. In the Lockwitzbach the mean risk doubles during rain events, from 0.0152 to 0.0311. During baseline conditions the lowest value of any sample is still more than twice that of the risk threshold, with 0.0028. During rain events the lowest ΣTU_{Crus} is still one order of magnitude higher than the threshold.

Within the Geberbach the mean risk is more than four times higher during rain events than during baseline conditions, going from 0.0153 to 0.0651. The highest event sample is two orders of magnitude above the risk threshold and the lowest event sample is eight times higher than the threshold.

In the Launzige catchment the mean risk doubles during rain events going from 0.0328 to 0.0668. During baseline conditions the lowest value is still two and a half times that of the threshold value, and during rain events the lowest value is still more than five times that of the threshold value.

ΣTUs for fish

For the Lockwitzbach catchment the chronic risk threshold of 0.01 for fish is not exceeded in any of the baseline samples. During events this threshold is exceeded based on the mean ΣTUs_{Fish}, however it is not exceeded in half of the samples.

The Geberbach catchment does not exceed the threshold during baseline conditions based on the mean, however one sample did exceed it with the value 0.0177. During events this threshold is exceeded by roughly four times the threshold value, however two samples did remain below the threshold value.

In the Launzige catchment the threshold is not exceeded under baseline conditions based on the mean, however one sample did exceed the threshold with a value of 0.0224. During events the threshold is exceeded, and no samples are under the threshold value.

4.3.2 Biocides and pesticides dominate as toxicity drivers for all biological endpoints in all catchments.

For each catchment, bar graphs were made per biological endpoint for baseline and event conditions such as shown in figure 20, indicating the compounds which had the highest average TU in that catchment. Corresponding pie charts such as in figure 21 were also created to show the percentage contribution of the compounds to the ΣTU. The complete set bar graphs and pie charts can be found in appendix D. One of the most prominent findings is that across all catchments and for all biological endpoints in both baseline and event samples, the majority of the highest TU contributions are coming from pesticides and biocides despite a diverse mix of compound classes being detected at the highest concentrations and despite the fact that biocides never contribute more than 3% to the top 100 compounds with the highest concentrations.

Toxicity drivers for algae

For algae, many of the pesticides and biocides which contribute the most to the sum TU are herbicides, among them dimethenamid, metazachlor, terbutryn, terbuthylazine, and atrazine. Herbicides are known to dominate the mixture toxicity towards algae (Tang and Escher, 2014). Dimethenamid is on average the top contributing compound in the Lockwitzbach baseline samples however it does not appear in highest contributing lists for the Geberbach or Launzige in baseline or event samples, so this could be due to one particularly high detect of 291 ng L^{-1} where it was otherwise only found at values below 50 ng L^{-1} or not at all. The fungicide kresoxim-methyl contributes highly in the Lockwitzbach and Launzige in both baseline and event samples, contributing 14% and 12% in the Lockwitzbach and 19% and 13% in the Launzige, respectively. Terbuthylazine contributes highly and is in the top 7 contributing compounds of all catchments in both baseline and event samples and it is the top contributing compound in the Launzige baseline samples contributing 28% to the ΣTU_{algae}.

Another group of compounds consistently contributing highly to the ΣTU_{algae} are the quaternary ammonium compounds (QACs). QACs are a type of biocides used as disinfectants and are often used in cleaning products. Didecyldimethylammonium is one of the highest contributing compounds appearing in the top 6 contributing compounds across all

catchments and being the top contributing compound in the Launzige event samples contributing 16% to the total. This is despite it being detected in each catchment at only up to 70 ng L^{-1} as QACs are known have a high toxicity to many species (Zhang et al., 2015).

Terbutryn is a herbicidal biocide belonging to the group of triazines, which are also used as pesticides. Its use as a herbicide has been banned in Germany due to its toxicity however it is used in façade paints and therefore is still found in surface waters in the ng L^{-1} range (Quednow and Püttmann, 2007). It was in the top 6 compounds contributing to ΣTU_{algae} of all catchments in both baseline and event samples, and in the Lockwitzbach event samples it was the top contributing compound with 22%.

The pharmaceutical orlistat which is commonly used as an antiobesity medication showed a very high contribution to the ΣTU_{algae} in the Lockwitzbach under event conditions and in the Geberbach under both baseline and event conditions. This high contribution has to be considered with some uncertainty, as experimental ecotoxicity data is lacking and therefore its baseline toxicity is prediction based on a QSAR prediction. For this compound the prediction is outside of the model's domain and therefore is not reliable. It is therefore hard to comment on the accuracy of the contribution of orlistat to the total toxicity of the mixture. The antibiotics clarithromycin and sulfamethoxazole also appeared to contribute significantly to the ΣTU_{algae} across all catchments, the most of any other pharmaceuticals.

The polymer additive 2,2'-Methylenebis(6-tert-butyl-p-cresol) contributes the most of any polymer additives. It has been assessed by ECHA under substance evaluation as a potential endocrine disruptor (ECHA, 2017). It contributes between 1-4% to the ΣTU_{algae} across most of the catchments.

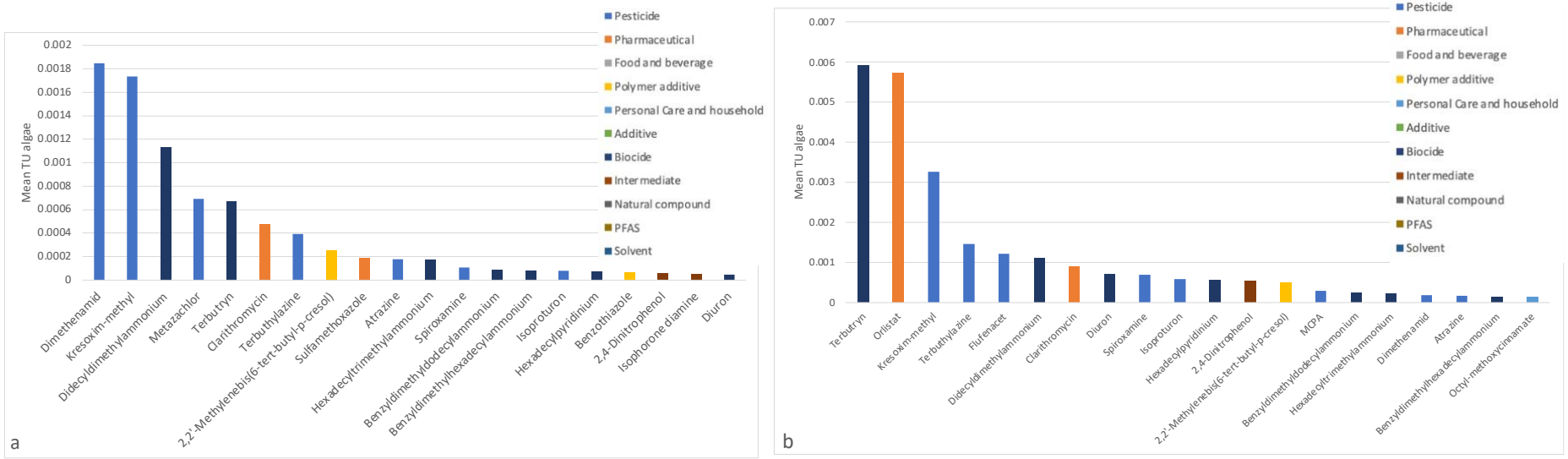

Figure 20: Bar graph showing the highest TU_{algae} of each compound detected across all Lockwitzbach samples for a: baseline and b: event.

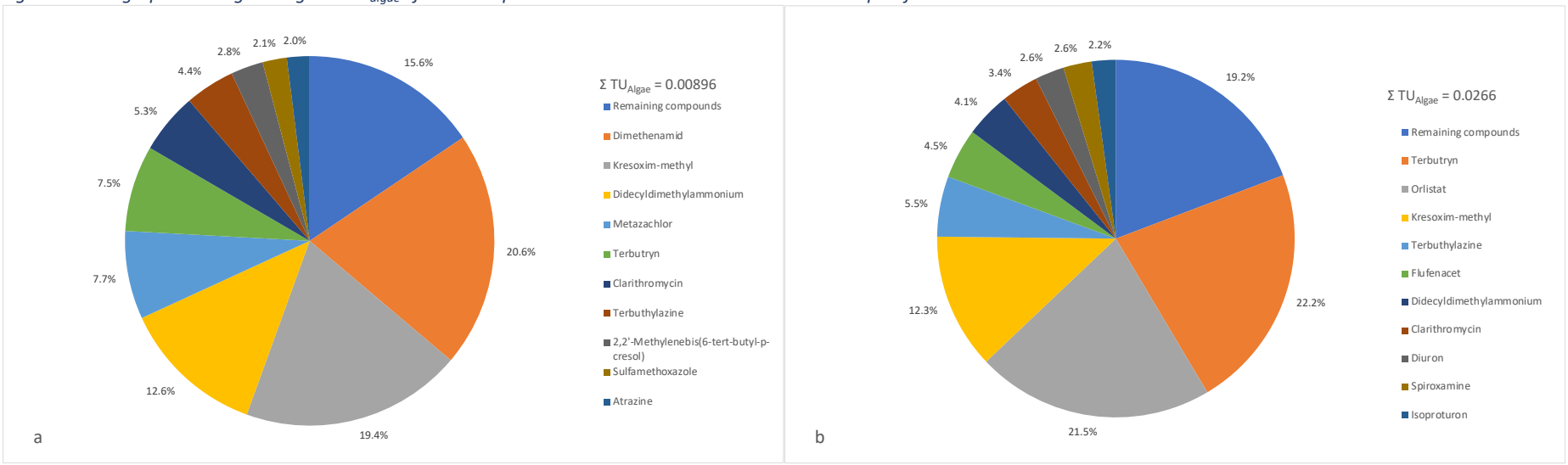

Figure 21: Pie chart showing the absolute value and percentage contribution to the ΣTU_{algae} of the top 10 contributing compounds in the Lockwitzbach for a: baseline and b: event samples.

Toxicity drivers for crustaceans

Many of the pesticides and biocides discussed in the previous section such as kresoxim-methyl, didecyldimethylammonium, and terbuthylazine were also amongst the top contributing compounds to the ΣTU_{crus}. For the crustaceans, insecticides such as diflubenzuron, imidacloprid, and the transformation product 3,5,6-trichloro-2-pyridinol also contribute heavily to the total toxicity of the mixture. In the Lockwitzbach event samples diflubenzuron is the top toxicity driver contributing 21% to the ΣTU_{crus}, in the Geberbach baseline samples the top driver imidacloprid contributes 40% to the ΣTU_{crus}, and in the Launzige event samples 3,5,6-trichloro-2-pyridinol is the top driver contributing 32% to the ΣTU_{crus}.

Orlistat contributes a large share to the ΣTU for crustaceans in the Lockwitzbach event and Geberbach baseline and event samples, however the same uncertainties discussed in the previous section remain. Orlistat aside, diclofenac is the highest contributing pharmaceutical for crustaceans contributing 21% and 10% in the Lockwitzbach baseline and event samples respectively, and 15% and 7% to the Launzige baseline and event samples respectively. Diclofenac is known to cause chronic toxicity at environmentally relevant concentrations (Parolini, 2020).

The polymer additive 2,2'-Methylenebis(6-tert-butyl-p-cresol) contributes the most of any polymer additives to the ΣTU_{crus} and contributes between 1-5% to the ΣTU_{crus} across most of the catchments. The intermediate 2,4-dichlorophenol appears to contribute significantly to the ΣTU_{crus} appearing as one of the top 16 drivers in the Lockwitzbach and the Launzige, and in the Lockwitzbach baseline it is the top driver contributing 36% to the total toxicity. 2,4-Dichlorophenol is a precursor for many chemicals and a transformation product of many pharmaceuticals and pesticides therefore could possibly be reaching surface waters from a variety of sources.

Toxicity drivers for fish

As for the algae and crustaceans, pesticides and biocides dominate the mixture toxicity towards fish, including previously discussed compounds such as kresoxim-methyl and didecyldimethylammonium. QACs in particular dominate here with the highest or second

highest driver being didecyldimethylammonium contributing up to 45% to the sum TU_{fish} across all catchments in both baseline and event samples. Other QACs such as Hexadecyltrimethylammonium, Hexadecylpyridinium, Benzyldimethyldodecylammonium, Benzyldimethylhexadecylammonium, and Benzyldimethyltetradecylammonium commonly appear across all catchments in the top 20 contributing compounds. Some QACs have been known to exhibit a synergistic effect at environmentally relevant concentrations (Di Nica et al., 2017).

The biocide carbendazim also contributes significantly to the ΣTU_{fish} consistently appearing in the top 10 contributing compounds across all catchments in both baseline and event samples. Carbendazim is a fungicide used pre- and post-harvest to prevent fungal diseases on various fruits, vegetables, and other crops, and embryonic exposure can lead to many adverse effects (Jiang et al., 2014).

The pesticide dimoxystrobin contributes significantly to the ΣTU_{fish} in the Launzige catchment in both baseline and event samples, being the third highest driver contributing 11% and being the sixth highest driver contributing 4% respectively. Dimoxystrobin is a strobilurin fungicide, a group of chemicals known to exhibit adverse effects such as DNA damage and sex-hormone disruption at sub-lethal concentrations on zebrafish (Wang et al., 2021).

5. Conclusion

A variety of compounds were detected across the three catchments mostly in the ng L^{-1} range, but with some of the highest concentration compounds reaching µg L^{-1} concentrations. Compounds were shown to belong to a variety of classes indicating inputs from various sources such as traffic and urban runoff (polymer additives and biocides), domestic wastewater (pharmaceuticals, personal care and household, and food and beverage), and agriculture (pesticides). The proportion coming from the different sources mostly was in line with the respective land use in each catchment, for example with high levels of traffic related polymer additives in the very urbanised Geberbach and high levels of pesticides in the more rural Launzige.

It was shown that for the Lockwitzbach, rain events do not majorly affect the overall pollutant composition, with mean concentrations remaining roughly the same between the baseline and event samples and variation increasing only slightly for some compound classes during rain events. The Geberbach was shown to exhibit typical pollutant dynamics during rain events with mean concentrations and variation increasing compared to baseline conditions, indicating that rain events do significantly impact the pollutant composition of this catchment. The Launzige showed little difference in mean concentrations and variation for some compound classes and some even showed higher concentrations in the baseline samples; however, there was a limited number of samples for this catchment.

All catchments show a clear increase in untreated wastewater inputs during rain events. The Lockwitzbach has the lowest relative inputs of untreated wastewater under both baseline and event conditions, whereas the Geberbach showed the highest relative inputs under both baseline and event conditions. Untreated wastewater is known to bring higher concentrations of certain micropollutants such as pharmaceuticals and personal care products into surface waters. Reducing the quantity of untreated wastewater reaching these catchments therefore would be a beneficial step to improving the water quality of these catchments.

The risk assessment using TUs revealed that there is a clear increase in risk during rain events in every catchment, and that for every biological endpoint the risk threshold is exceeded during rain events. For the crustaceans the risk threshold was exceeded in every sample for both baseline and event samples. On average, for algae the risk is not exceeded under baseline conditions in the Lockwitzbach or the Geberbach but is exceeded in the Launzige, and for fish the risk is not exceeded during baseline conditions in any catchment. There were however some baseline samples in which the risk was exceeded, therefore continued monitoring in these catchments would be of benefit to accurately assess how frequently the risk threshold is exceeded or to be aware if the situation worsens.

Analysis of the toxicity drivers in the samples showed that for all three catchments the main compounds contributing to the toxicity of the mixture are pesticides and biocides, in particular the QACs. Additionally, for the algae the herbicides were of particular significance and for the crustaceans the insecticides. This shows that the inputs of pesticides and biocides

in all three of the catchments is more significant than the inputs of traffic related polymer additives and untreated wastewater compounds. Therefore, to improve the quality of the water across these catchments, as it is the goal under the European WFD, efforts should be focussed towards reducing the quantities of pesticides and biocides reaching these streams.

6. References

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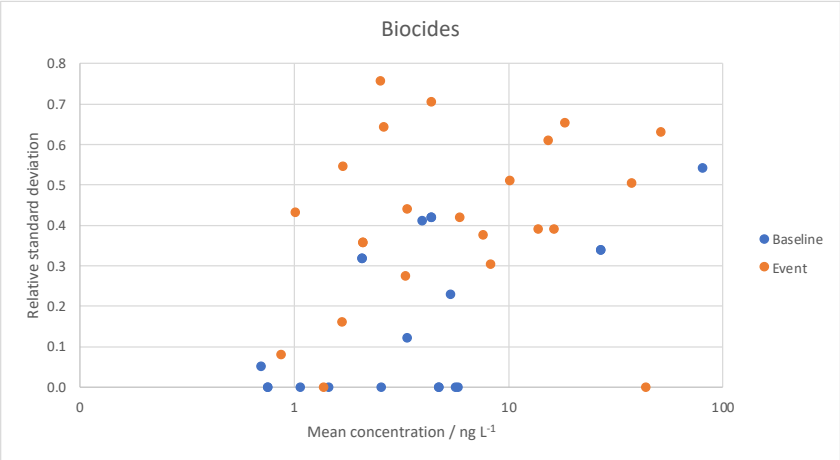
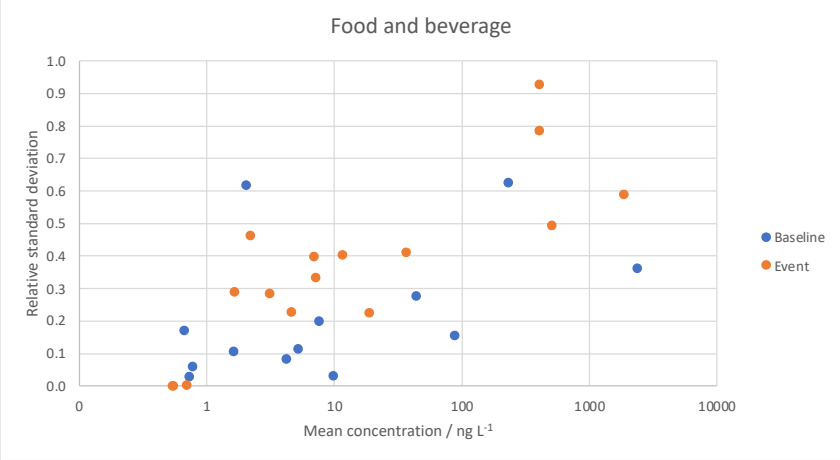
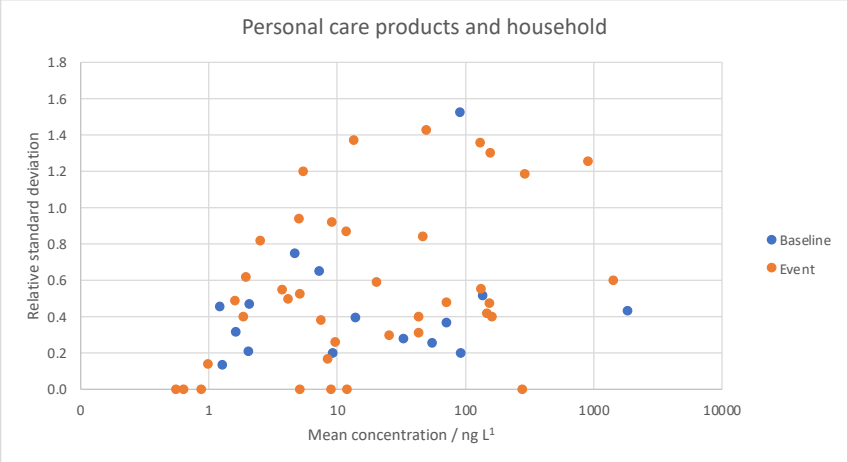
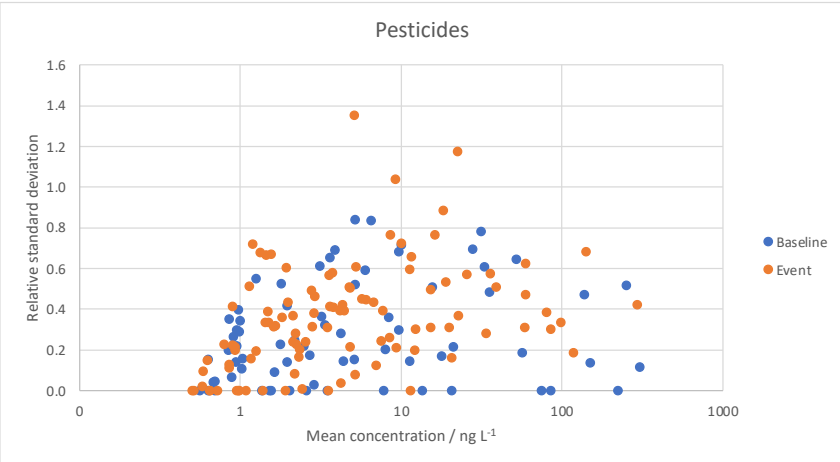
Appendix

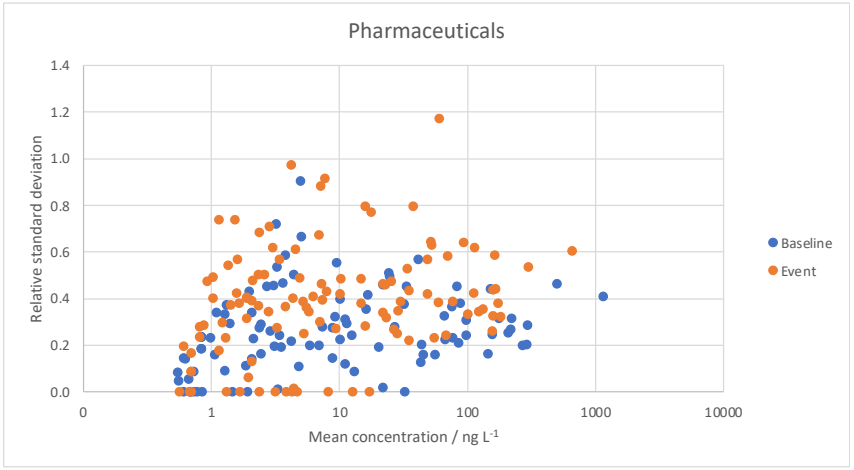
A: Scatter graphs showing mean concentration vs relative standard deviation for baseline and event samples in the Lockwitzbach catchment, per compound class.

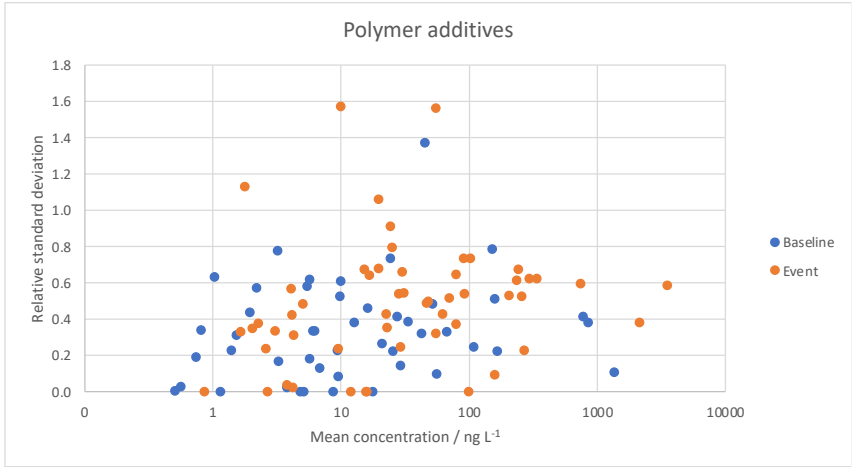




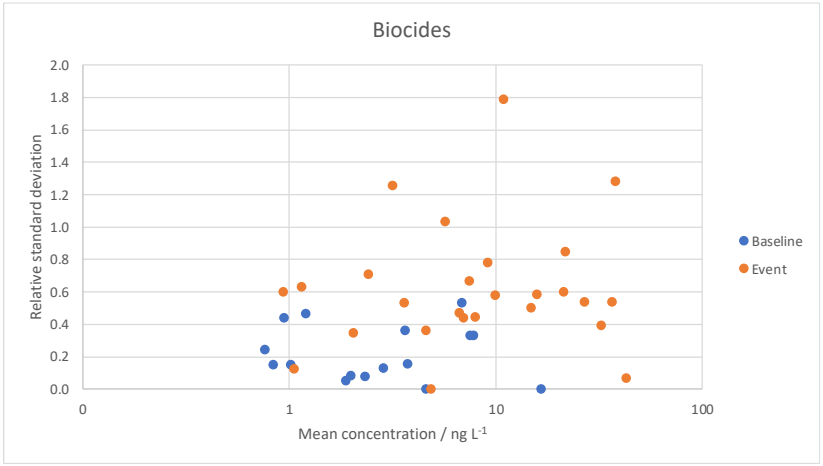


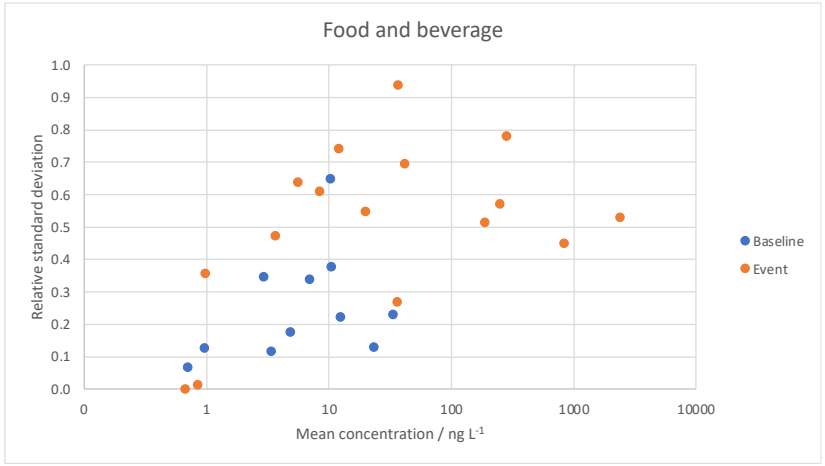


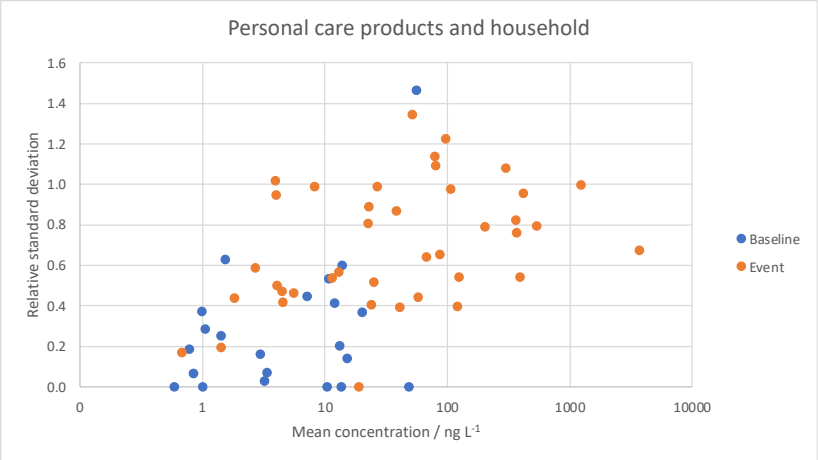


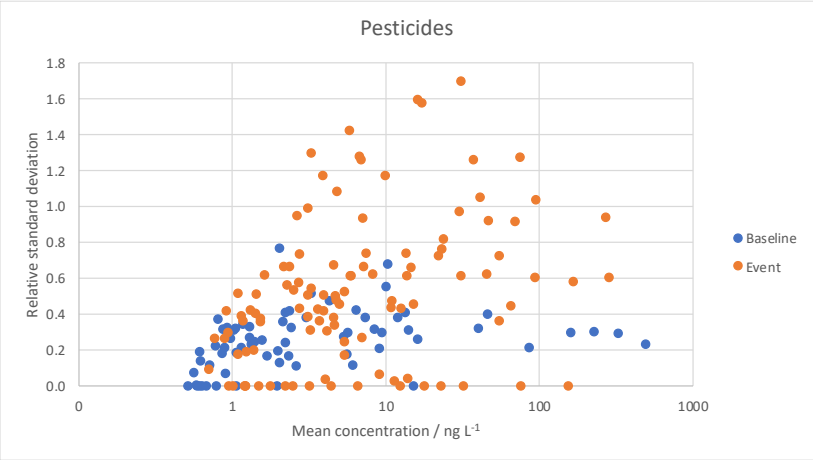


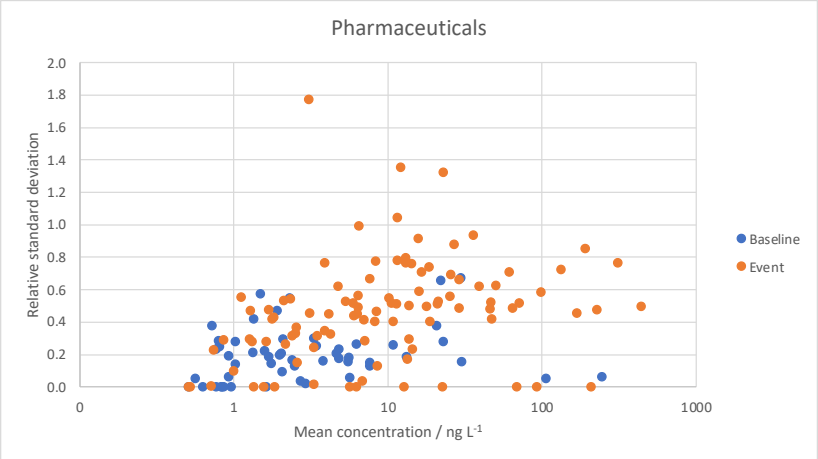
Appendix B: Scatter graphs showing mean concentration vs relative standard deviation for baseline and event samples in the Geberbach catchment, per compound class.

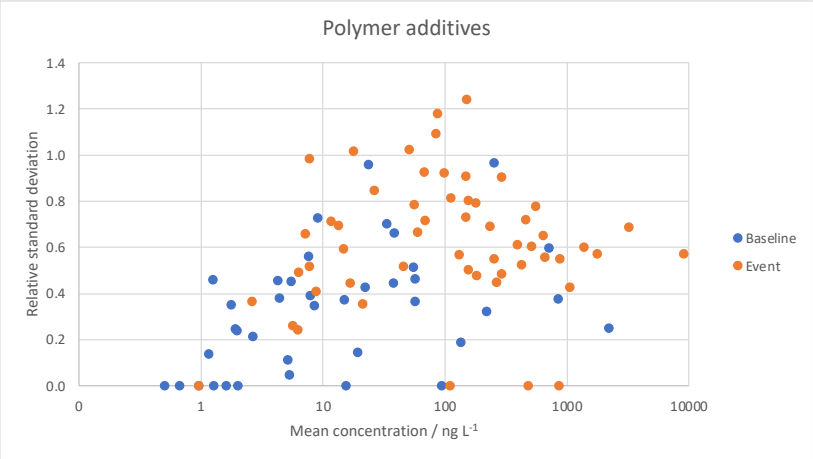




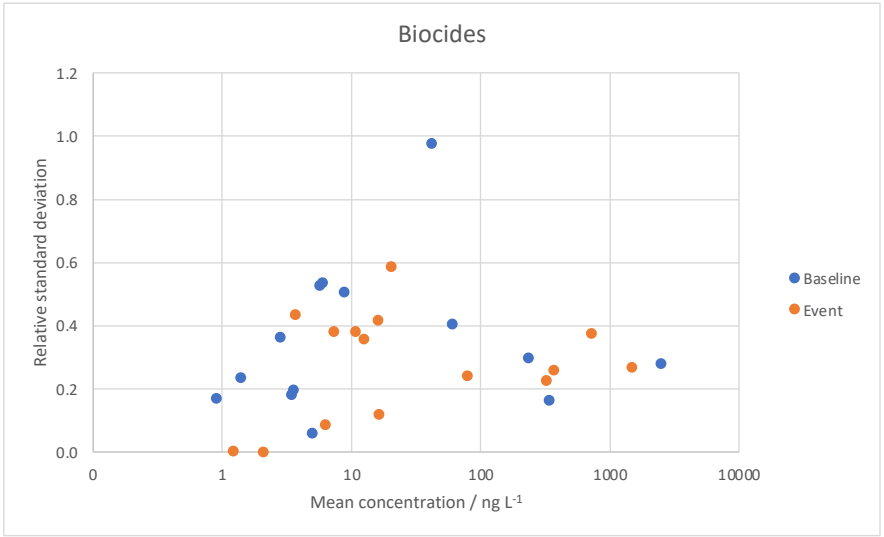

Personal care products and household
Relative standard deviation
Mean concentration / ng L-1
Baseline
Event

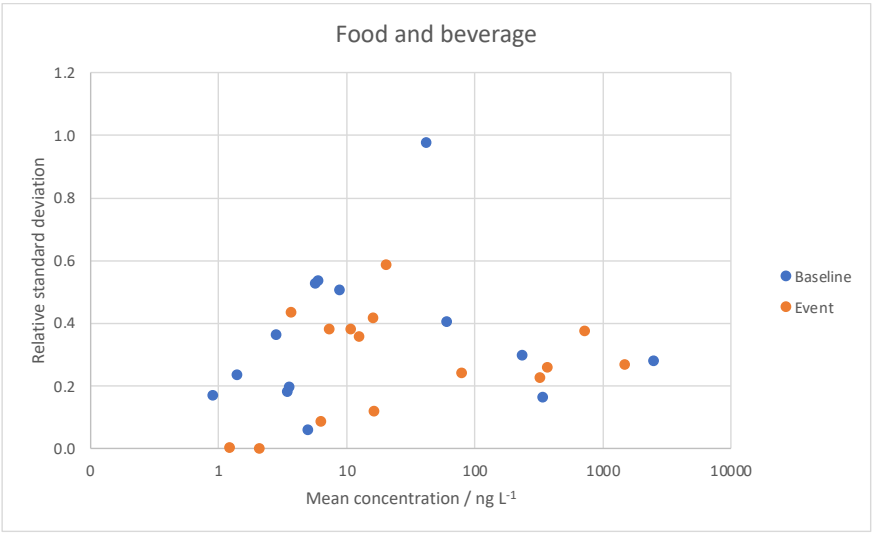

Pesticides
Relative standard deviation
Mean concentration / ng L-1
Baseline
Event

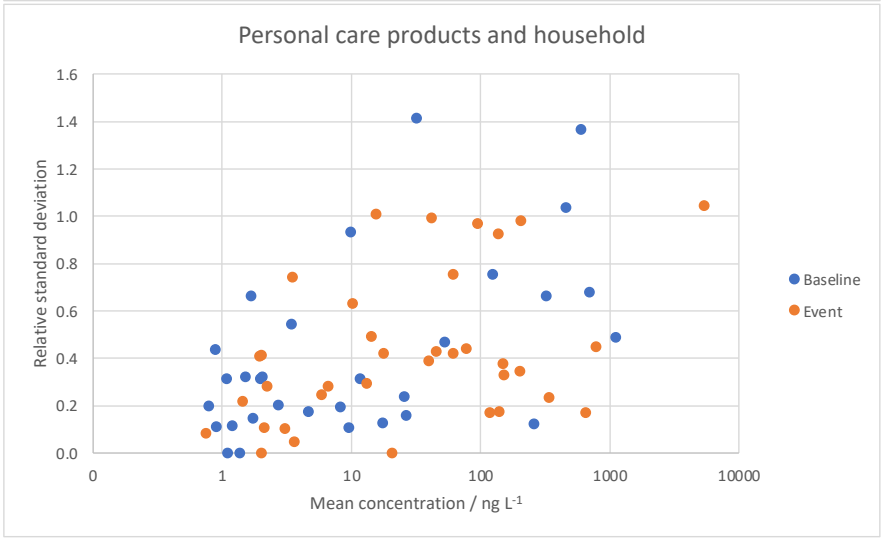

Pharmaceuticals
Relative standard deviation
Mean concentration / ng L-1
Baseline
Event

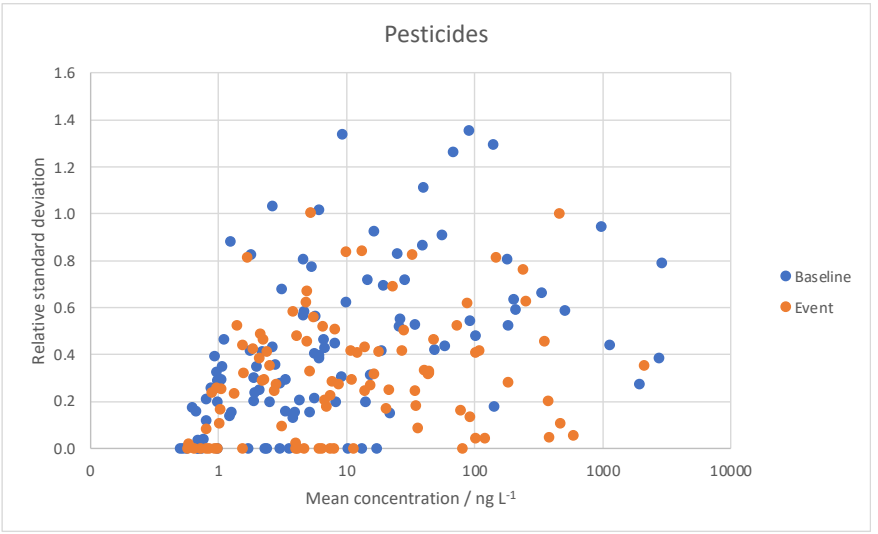

Polymer additives
Relative standard deviation
Mean concentration / ng L-1
Baseline
Event

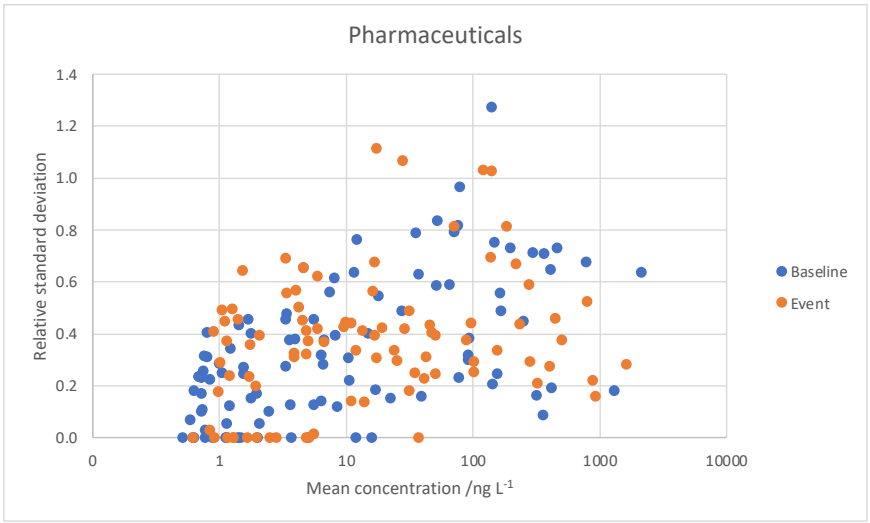
Appendix C: Scatter graphs showing mean concentration vs relative standard deviation for baseline and event samples in the Launzige catchment, per compound class.

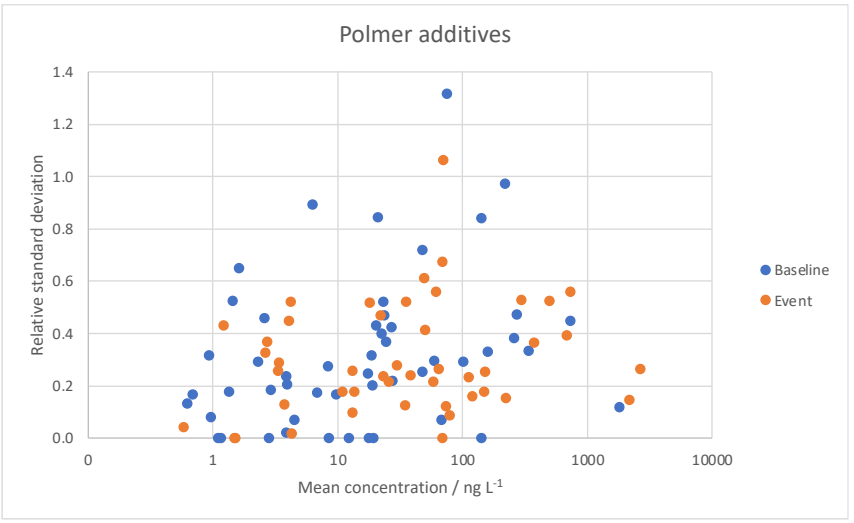






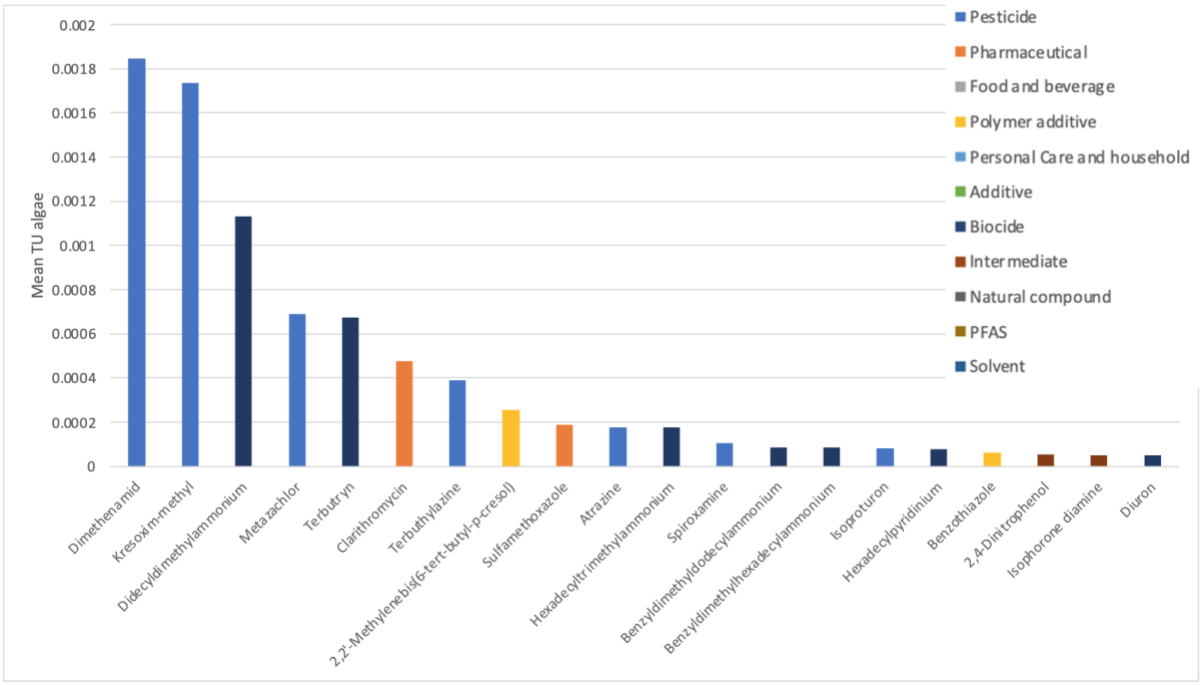


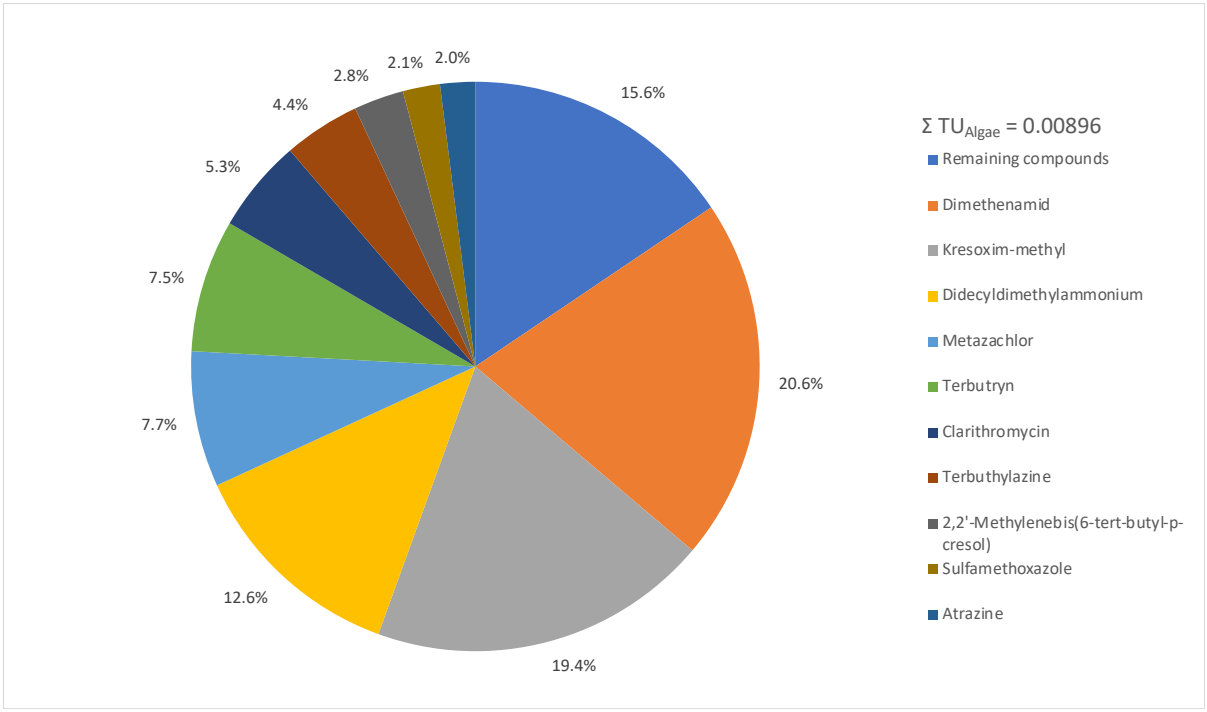

Pharmaceuticals
Relative standard deviation
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
10
100
1000
10000
Mean concentration /ng L-1
Baseline
Event


Polmer additives
Relative standard deviation
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
10
100
1000
10000
Mean concentration / ng L-1
Baseline
Event

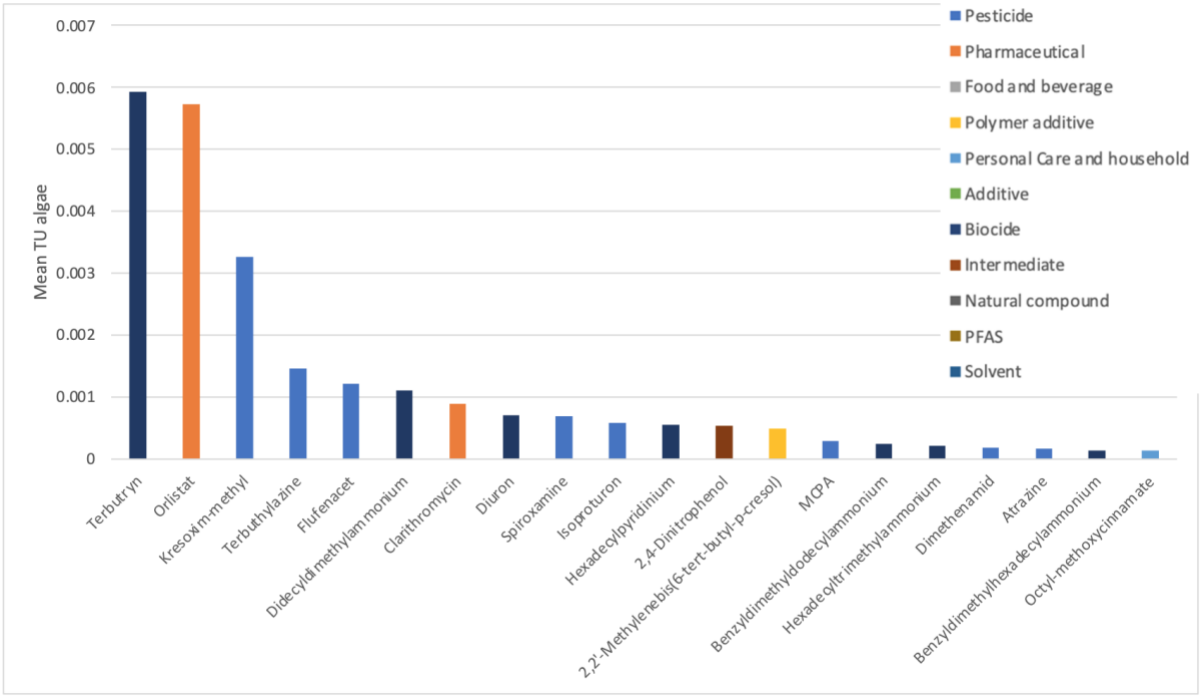
Appendix D: Bar graphs showing the highest $TU_{A,C,F}$ of each compound detected in each catchment, with corresponding pie charts showing the percentage contribution to the overall toxicity of the mixture

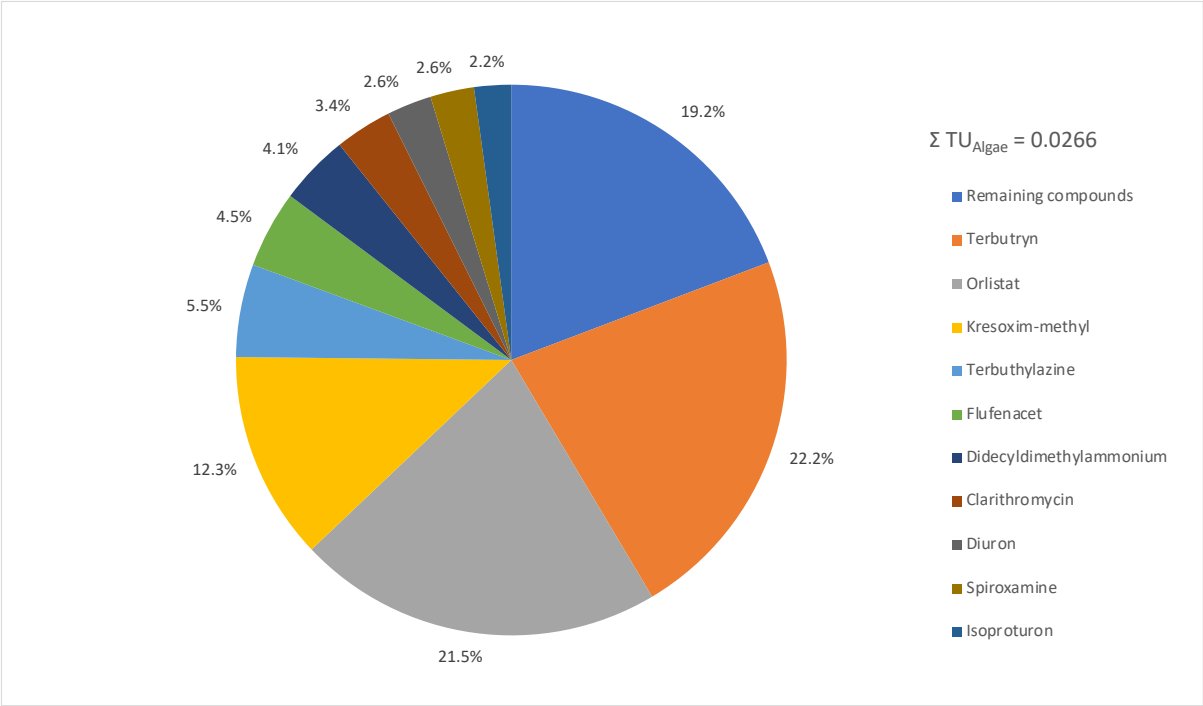
D.1 Lockwitzbach toxicity drivers baseline TU_{Algae}



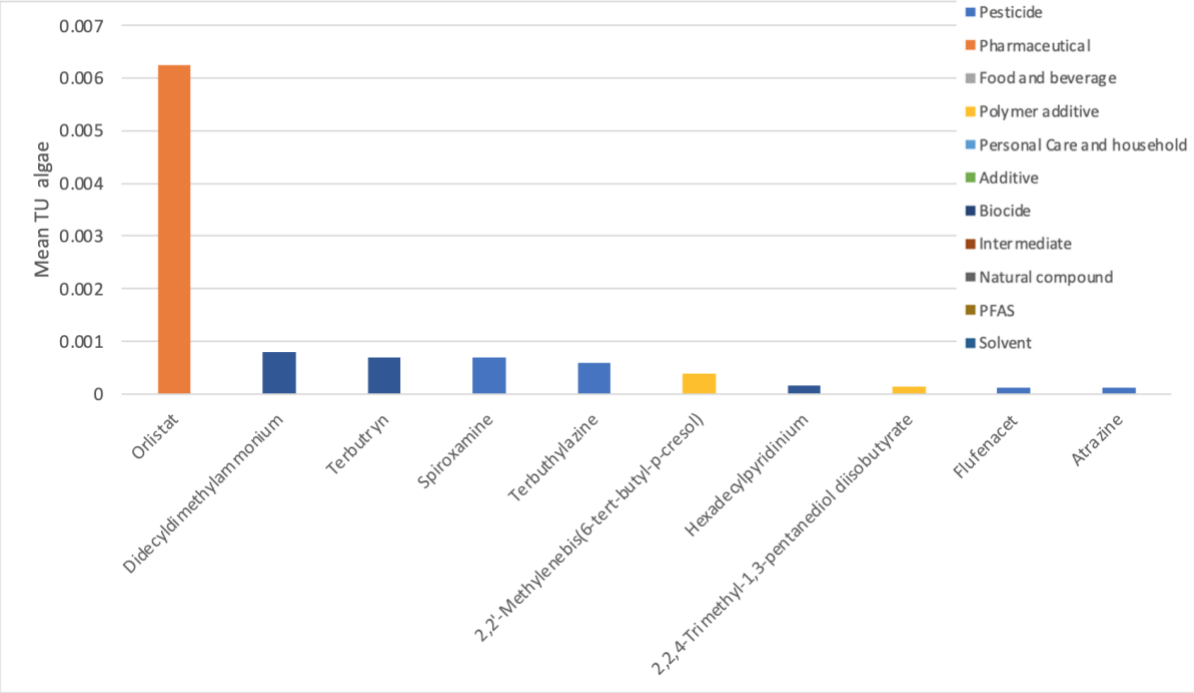


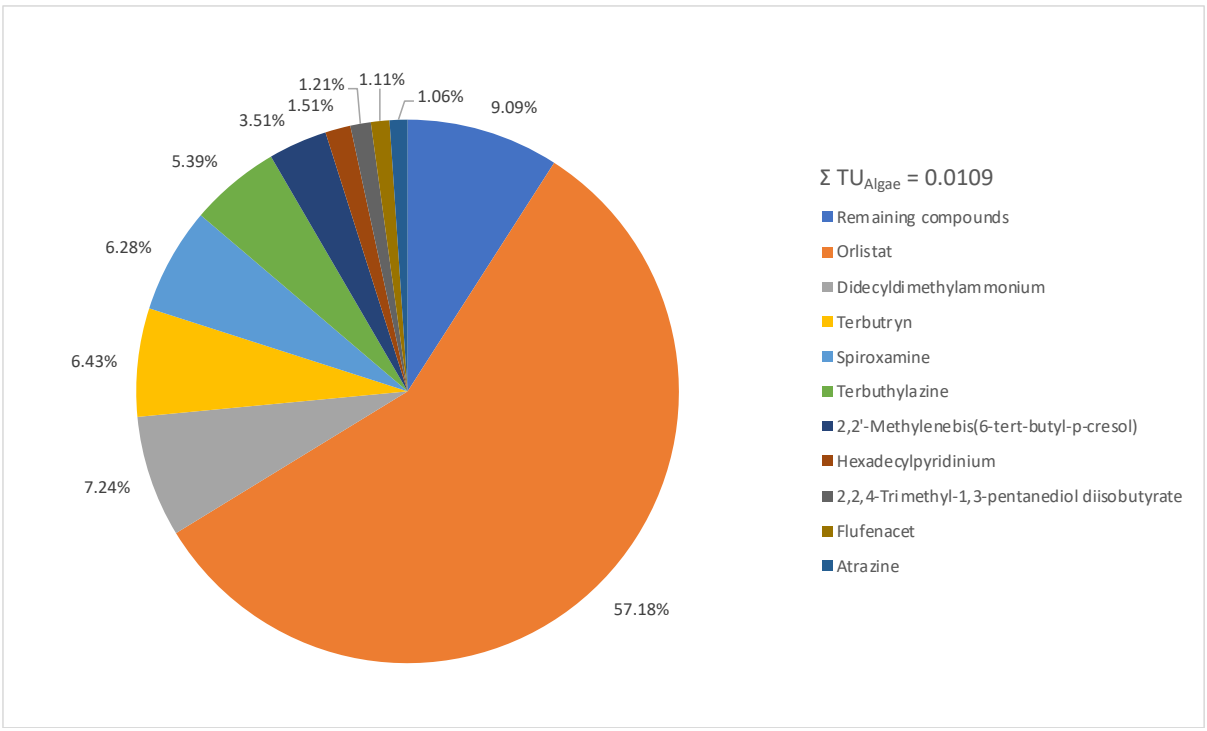
D.2 Lockwitzbach toxicity drivers event TU_{Algae}



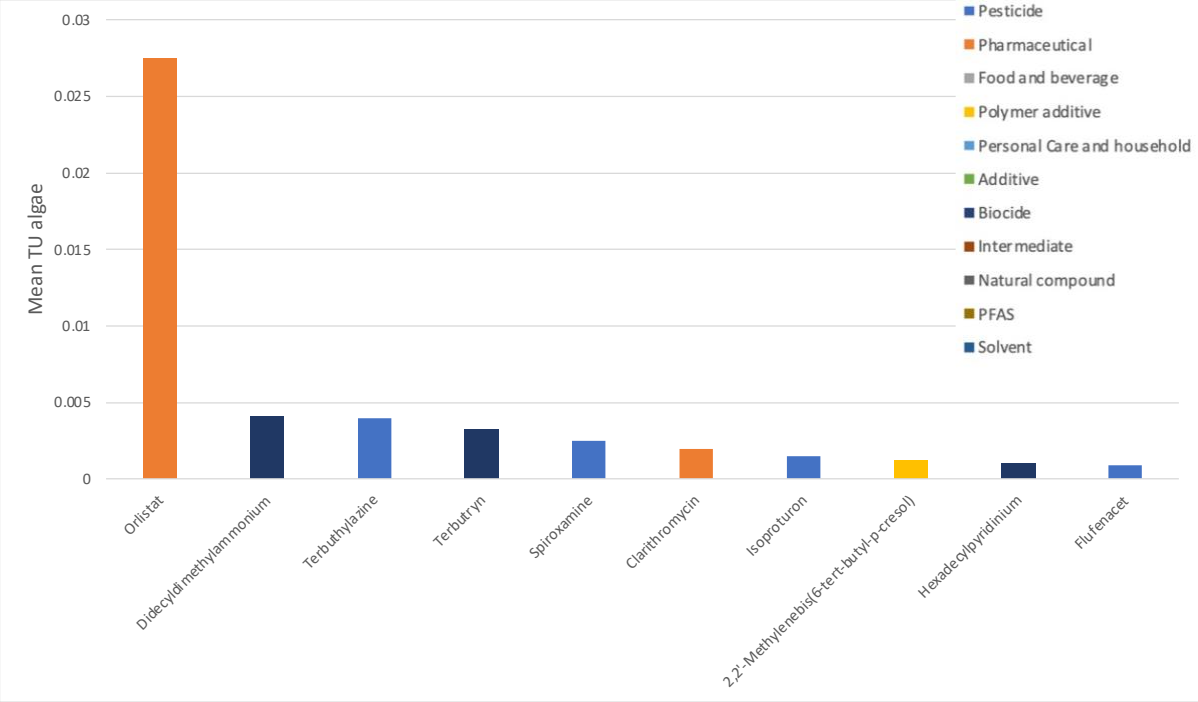


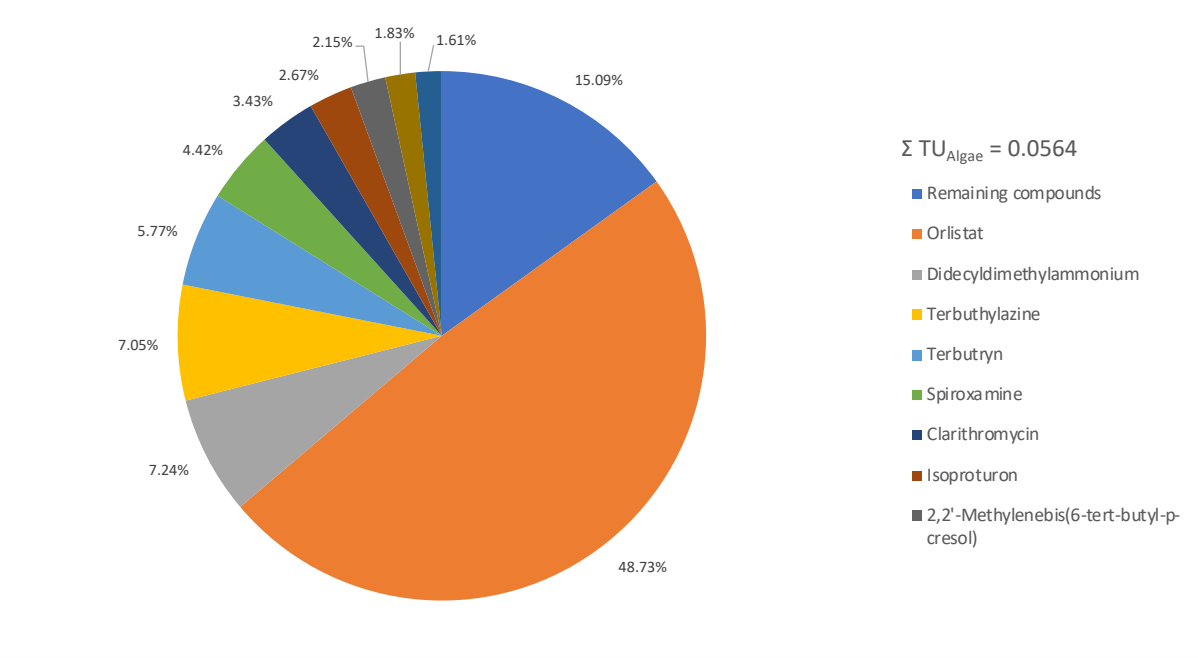
D.3 Geberbach toxicity drivers baseline TU_{Algae}



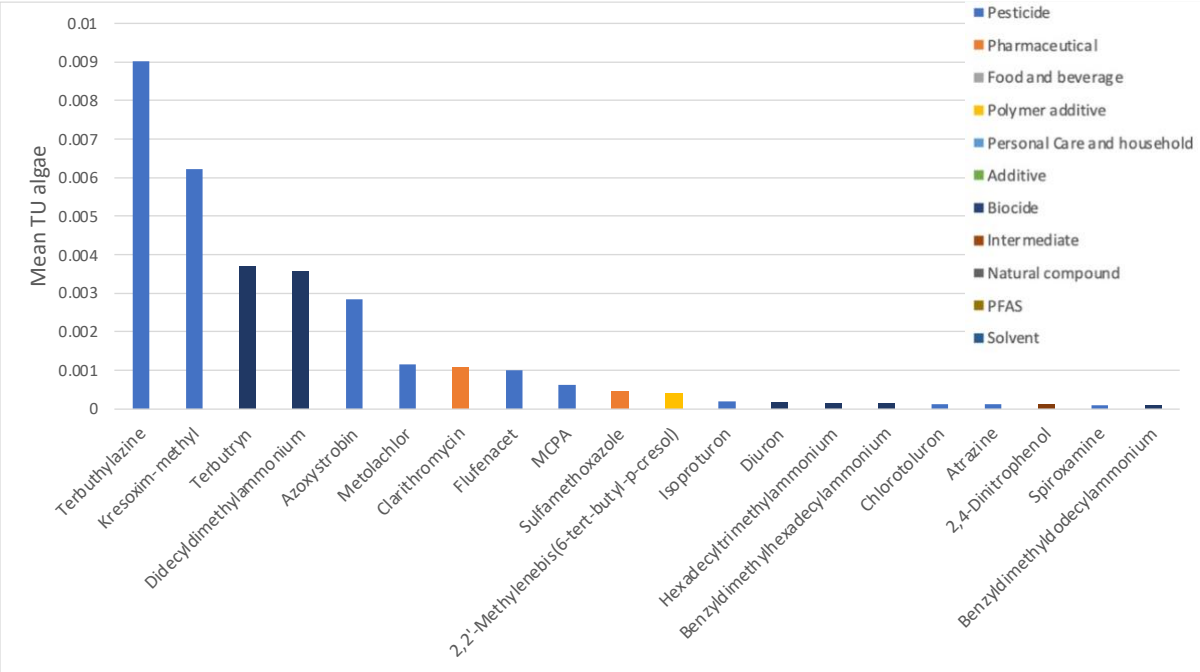


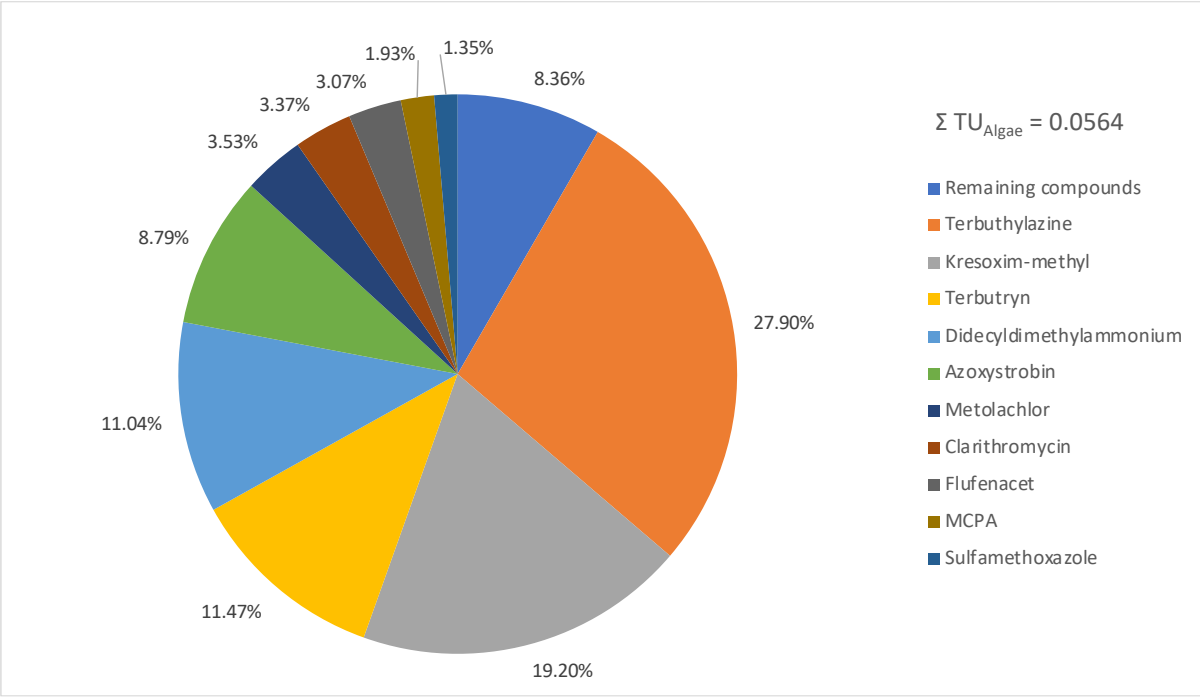
D.4 Geberbach toxicity drivers event TU_{Algae}



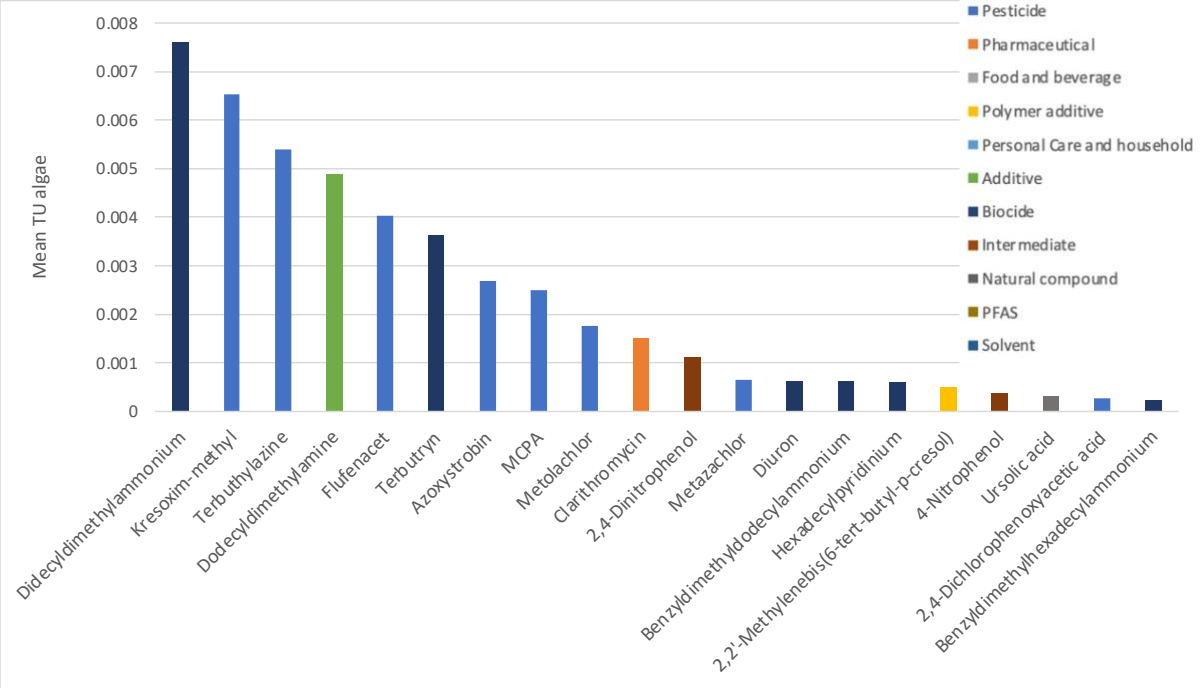


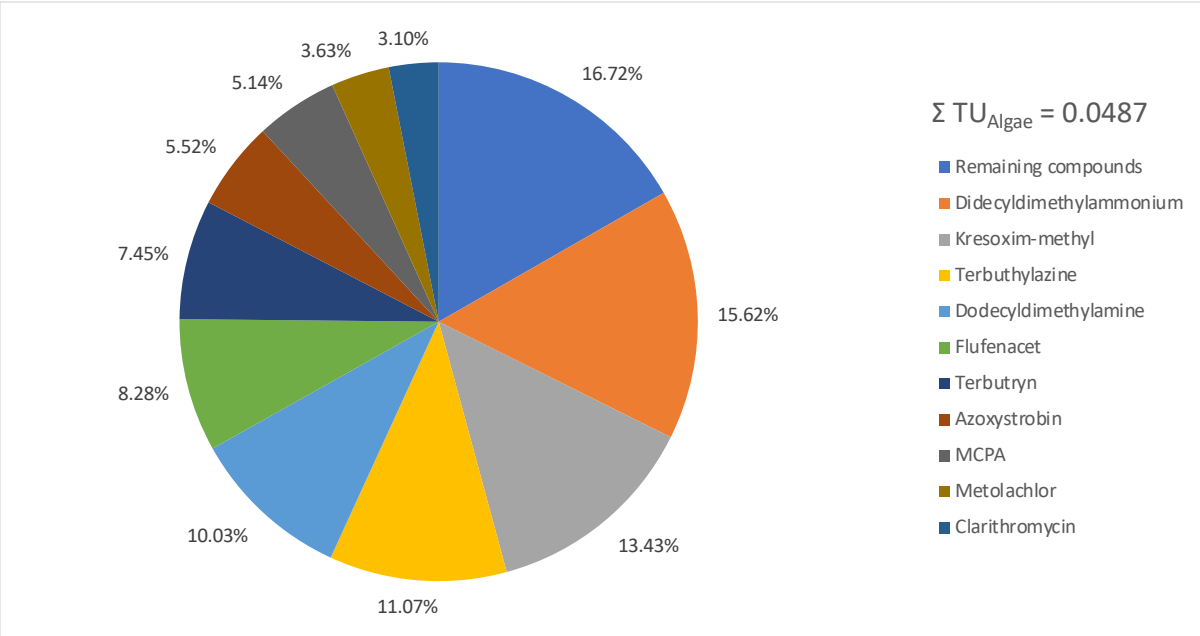
D.5 Launzige toxicity drivers baseline TU_{Algae}



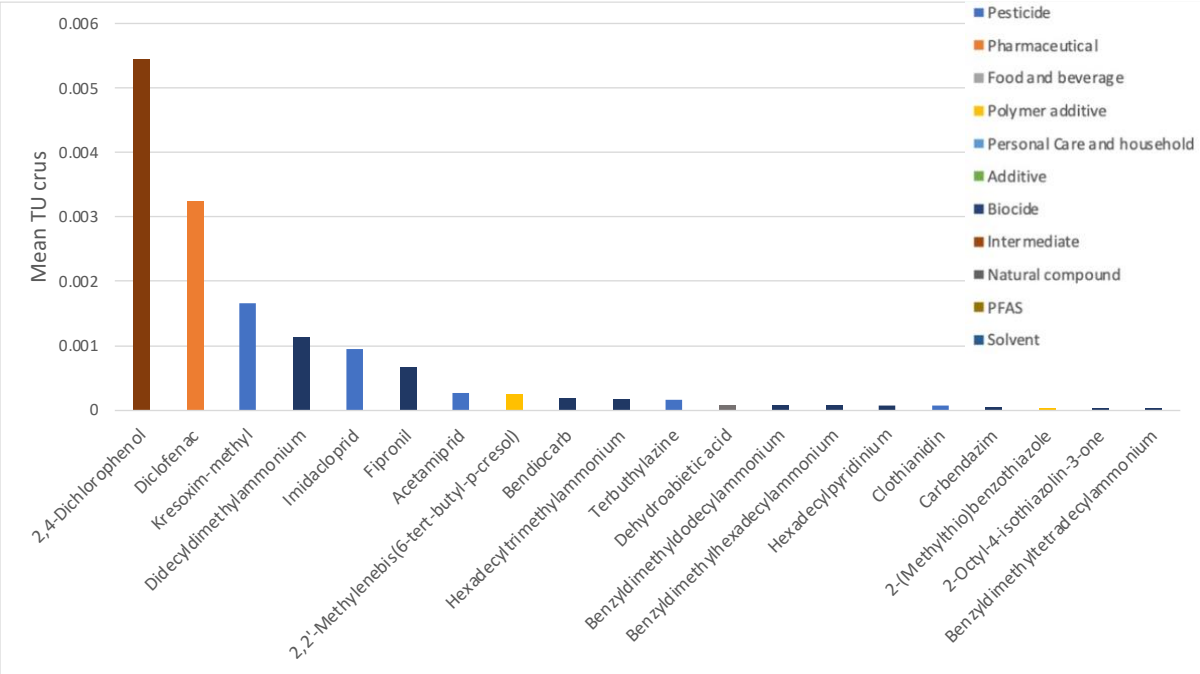


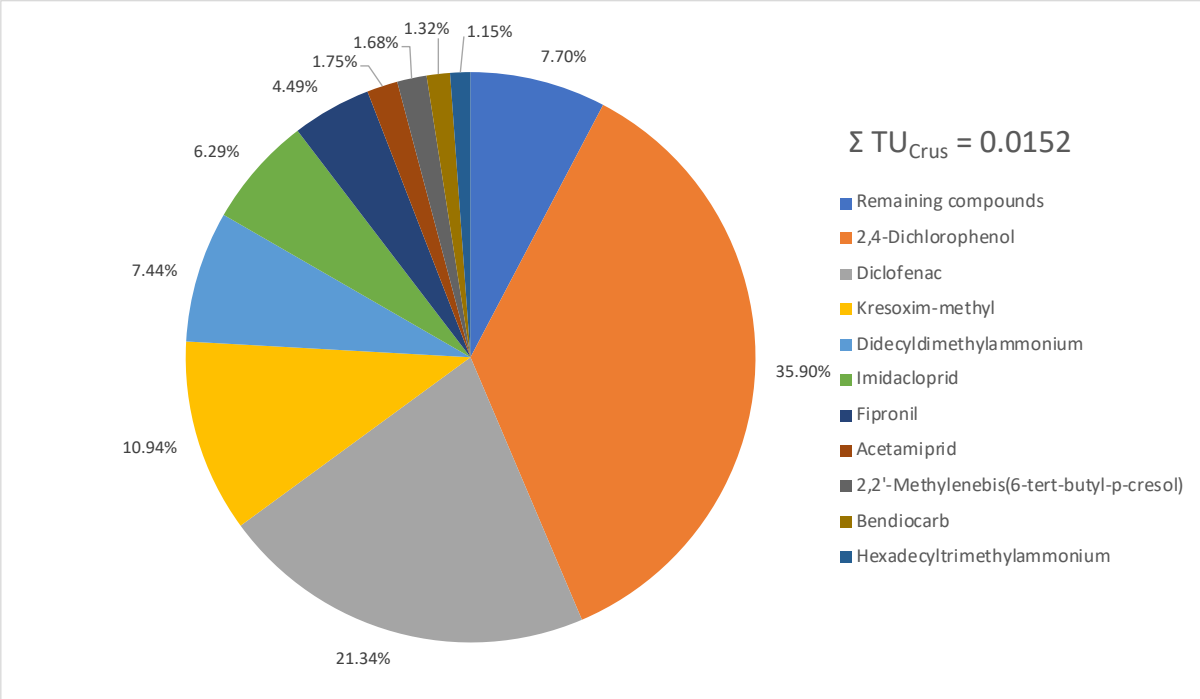
D.6 Launzige toxicity drivers event TU_{Algae}



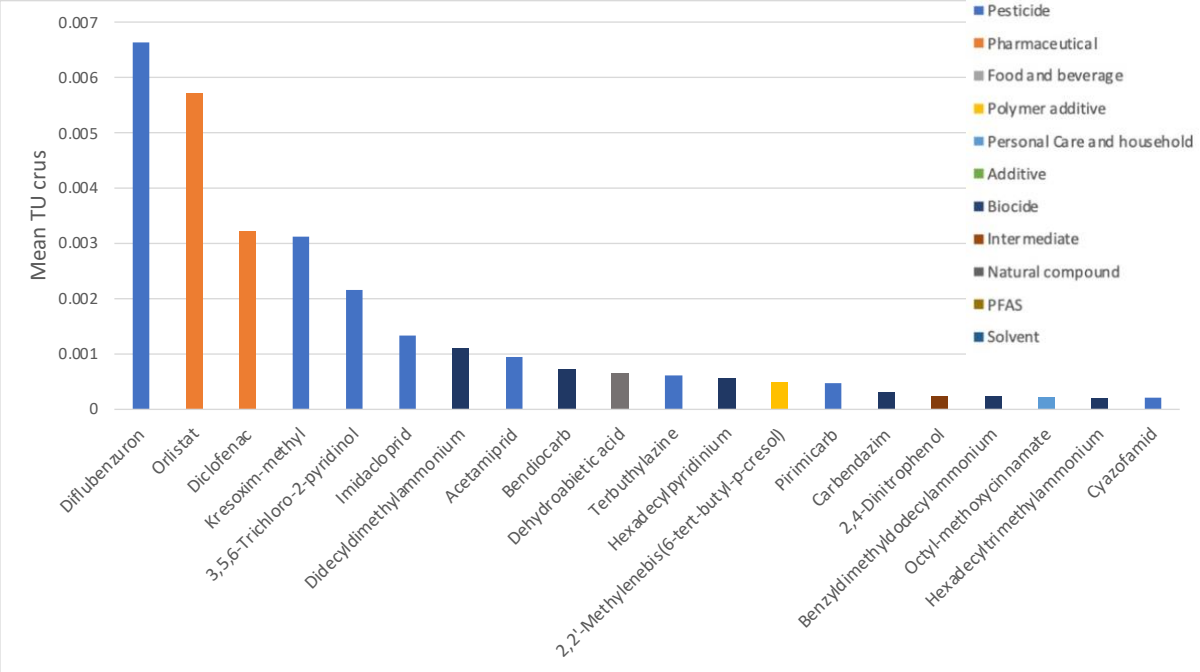


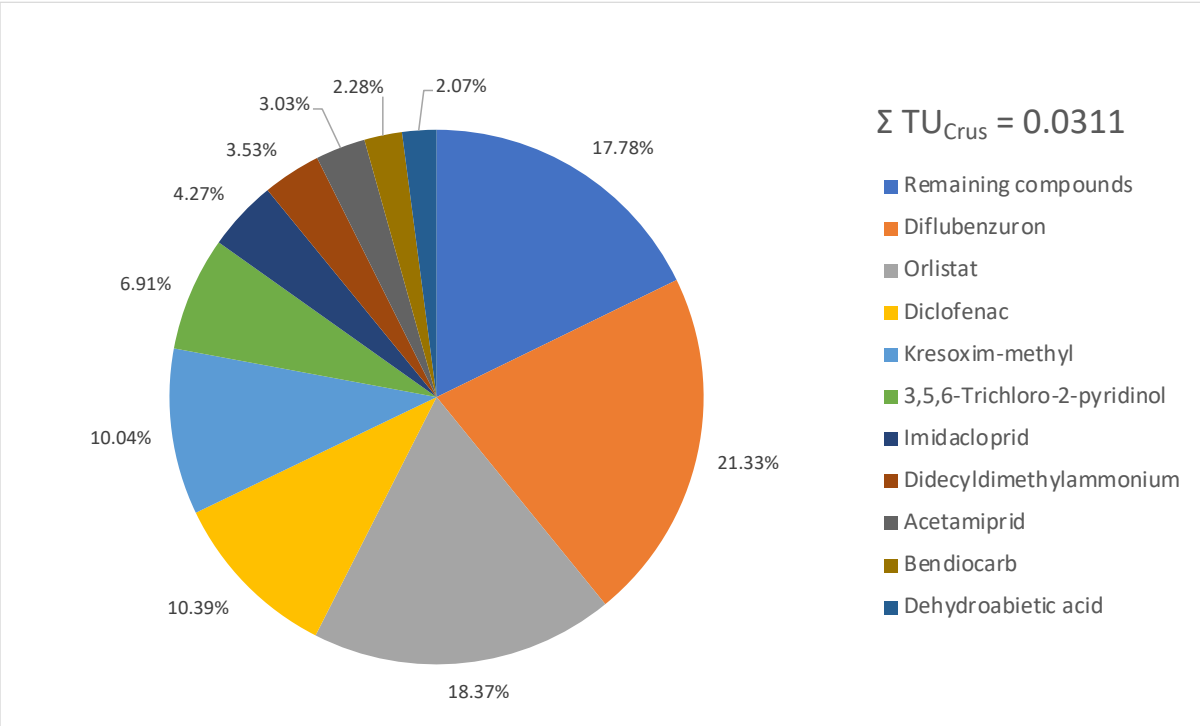
D.7 Lockwitzbach toxicity drivers baseline TU_{Crus}



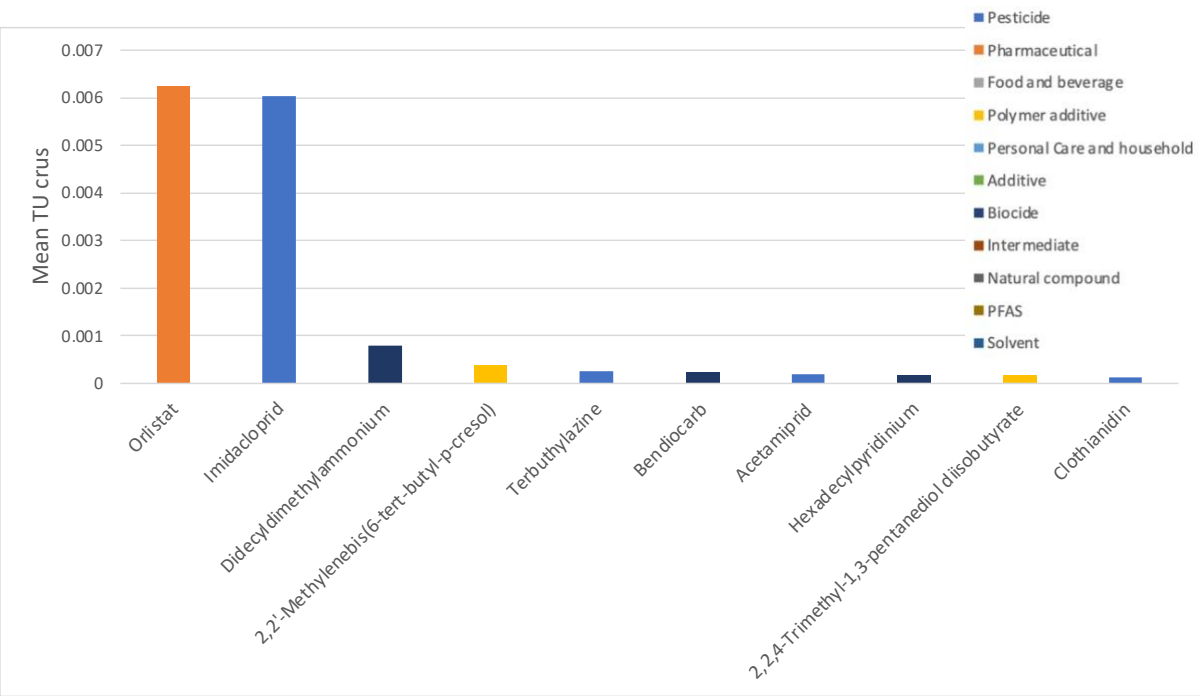


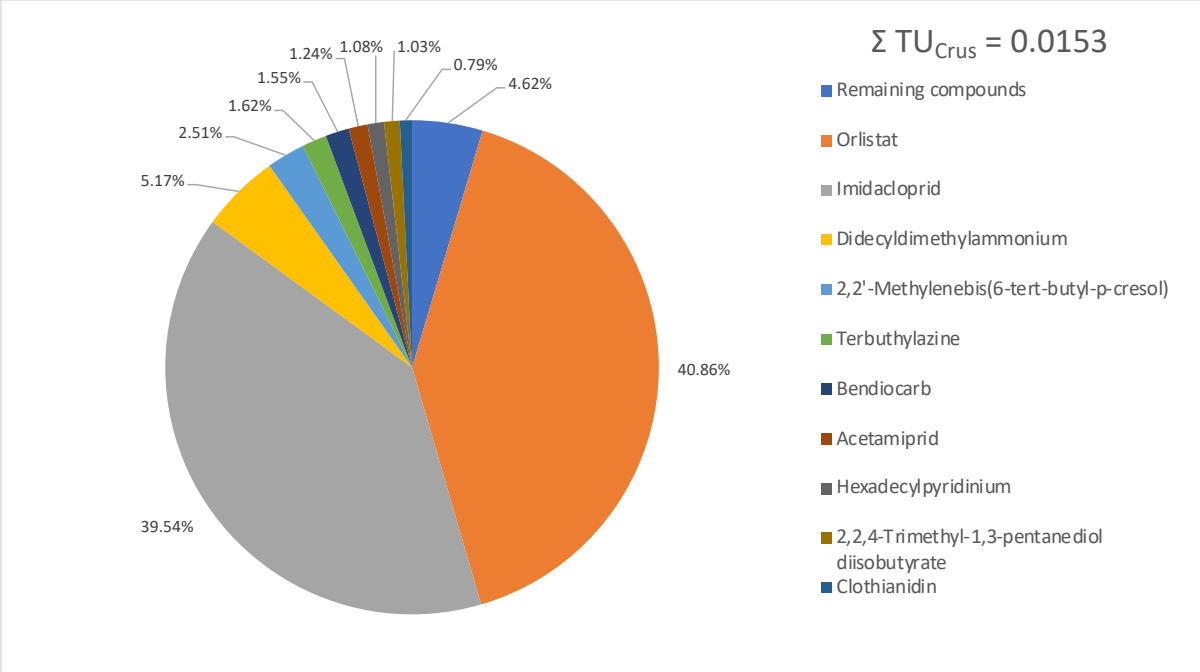
D.8 Lockwitzbach toxicity drivers event TU_{Crus}



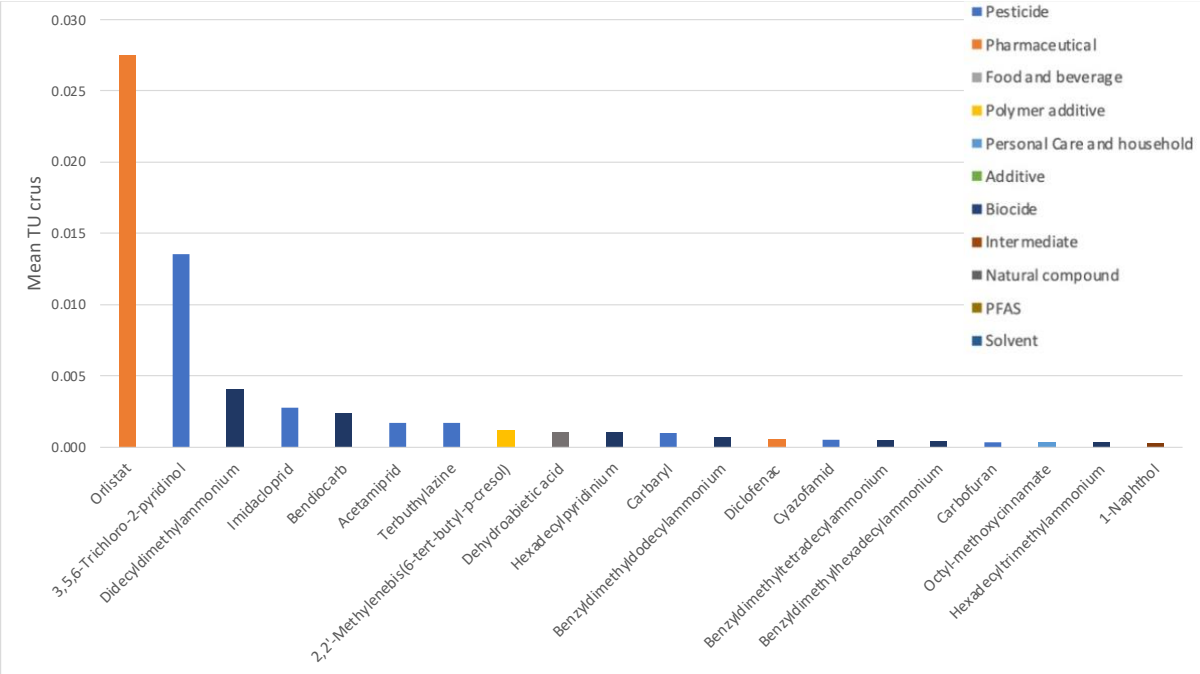


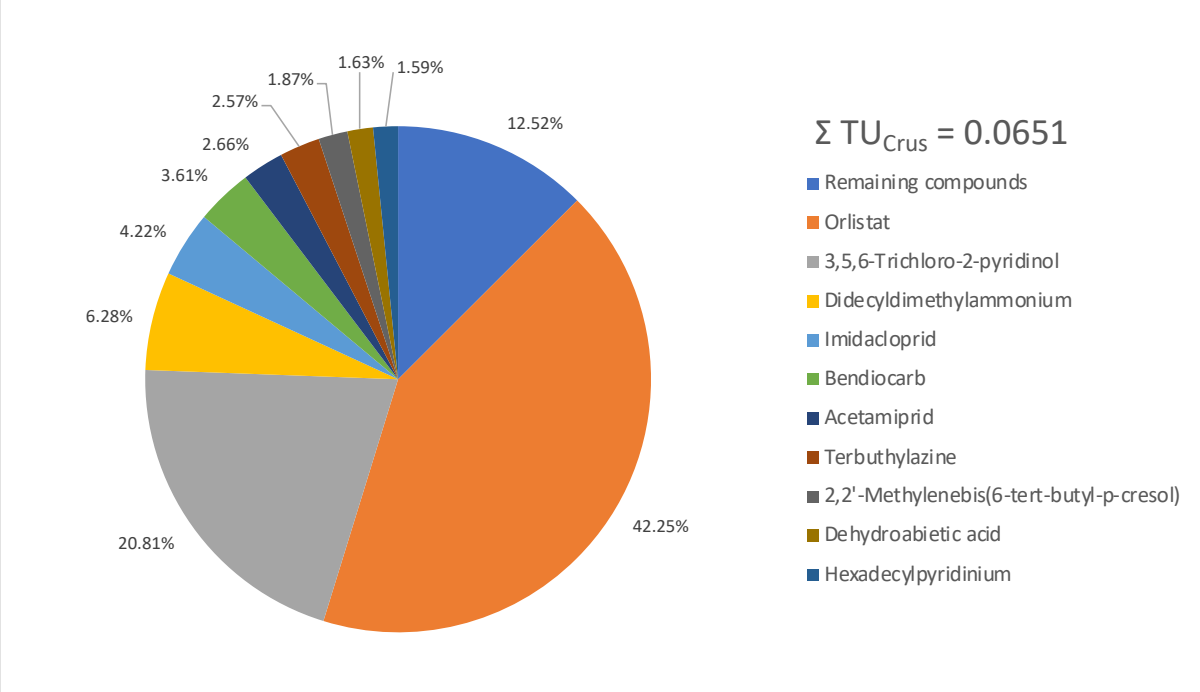
D.9 Geberbach toxicity drivers baseline TU_{Crus}



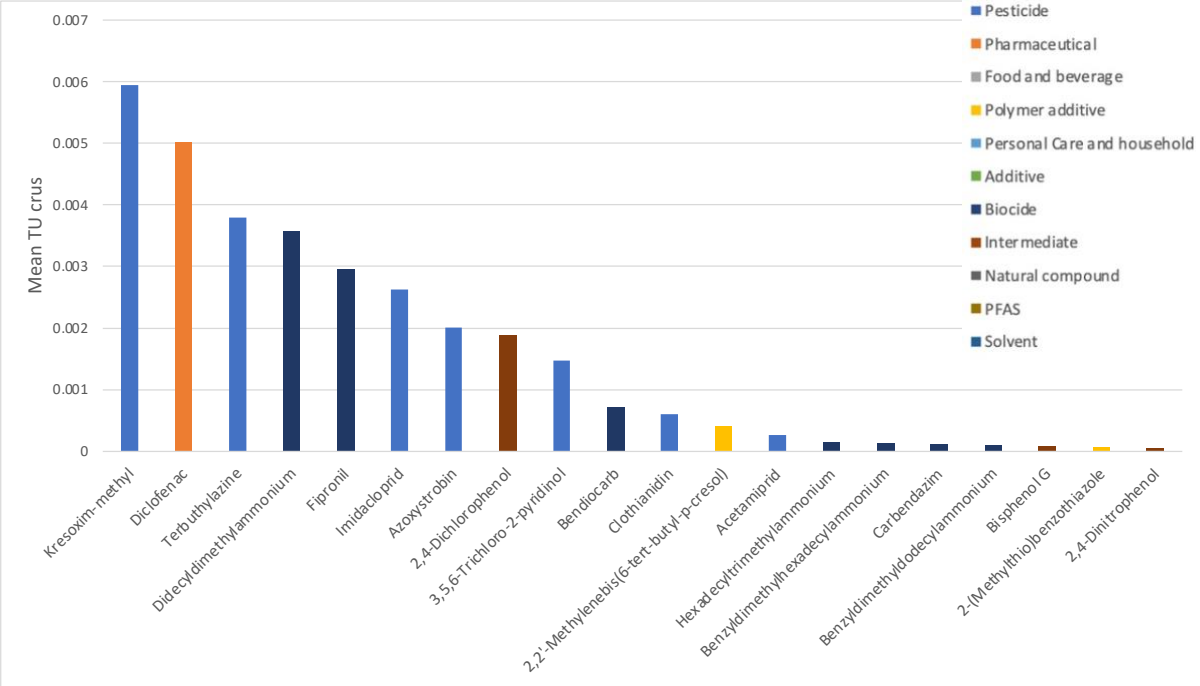


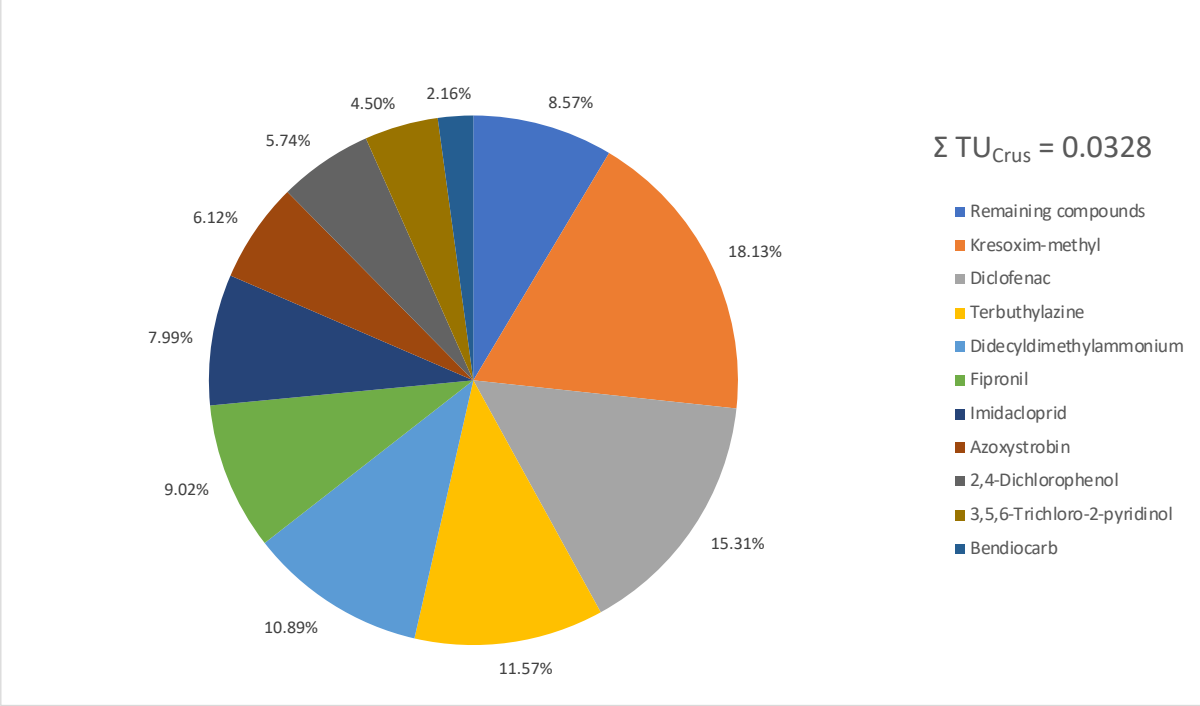
D.10 Geberbach toxicity drivers event TU_{Crus}



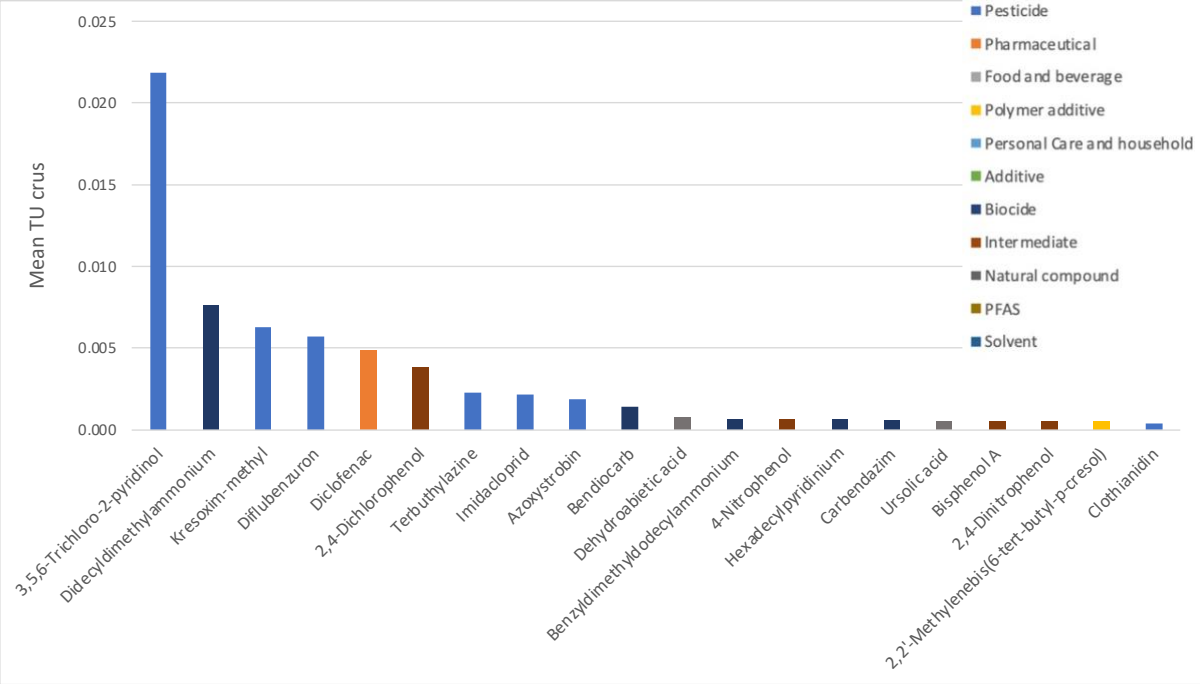


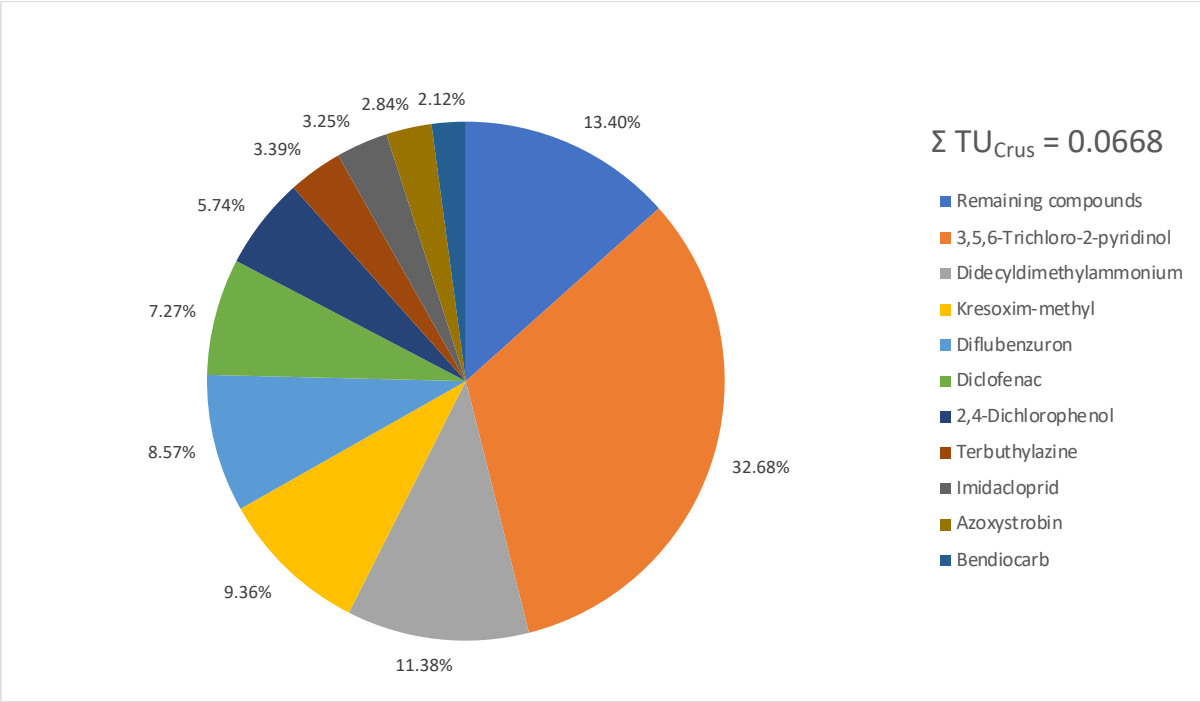
D.11 Launzige toxicity drivers baseline TU_{Crus}



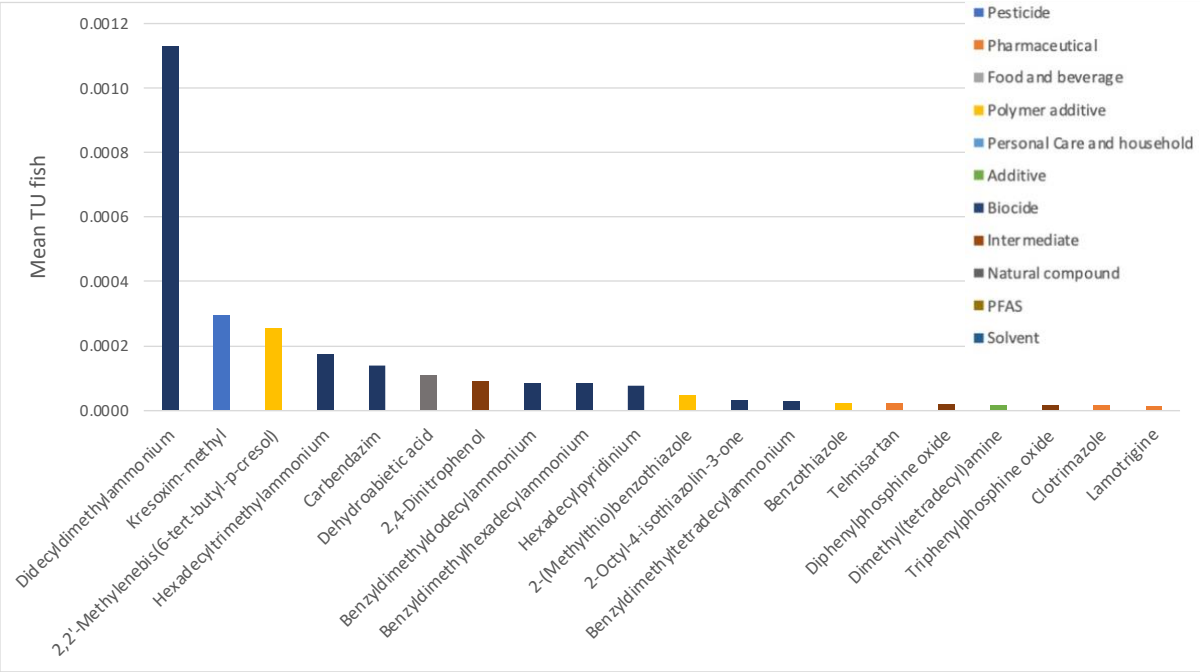


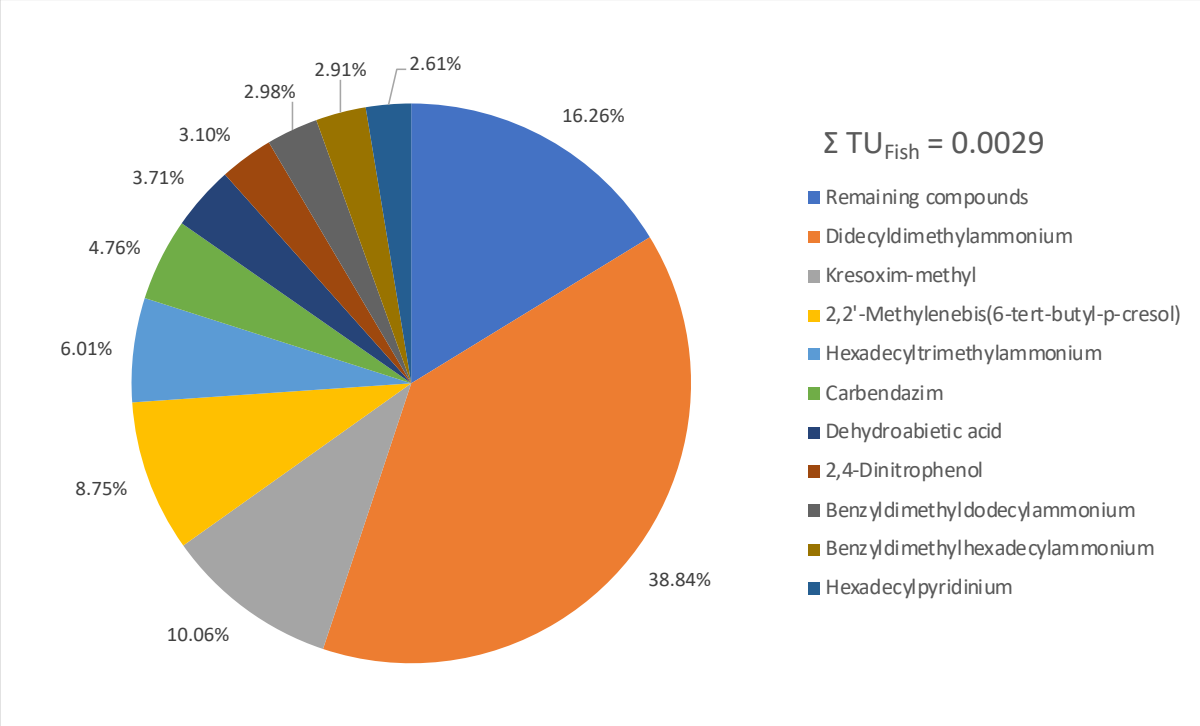
D.12 Launzige toxicity drivers event TU_{Crus}



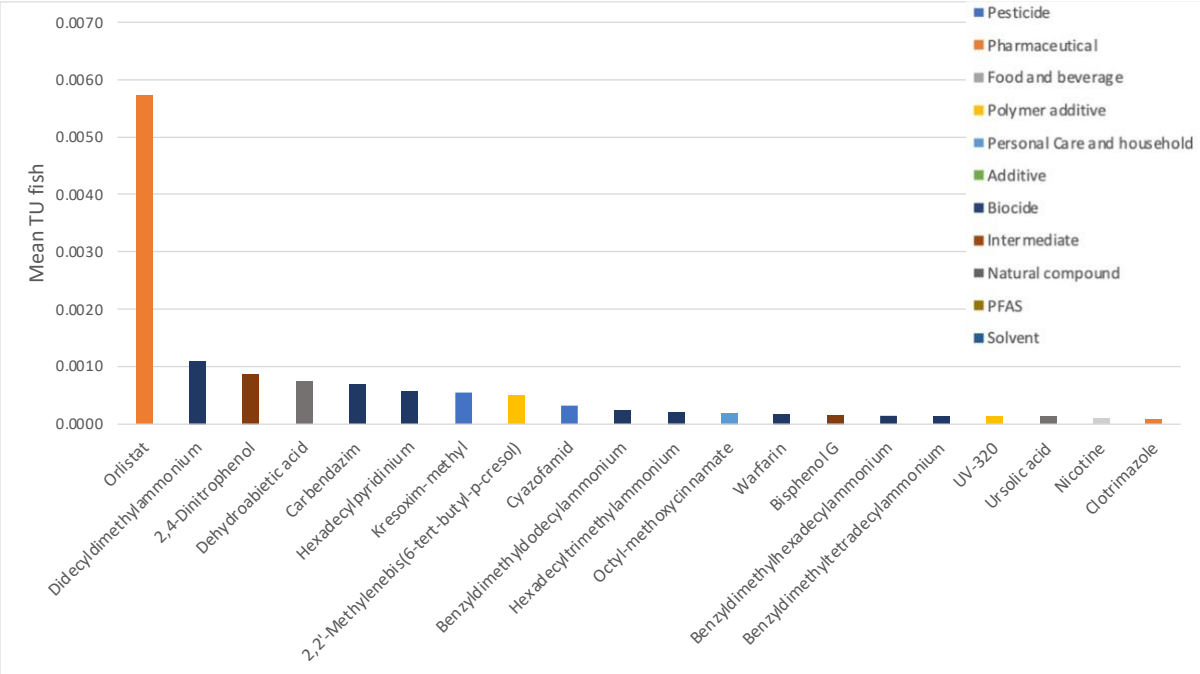


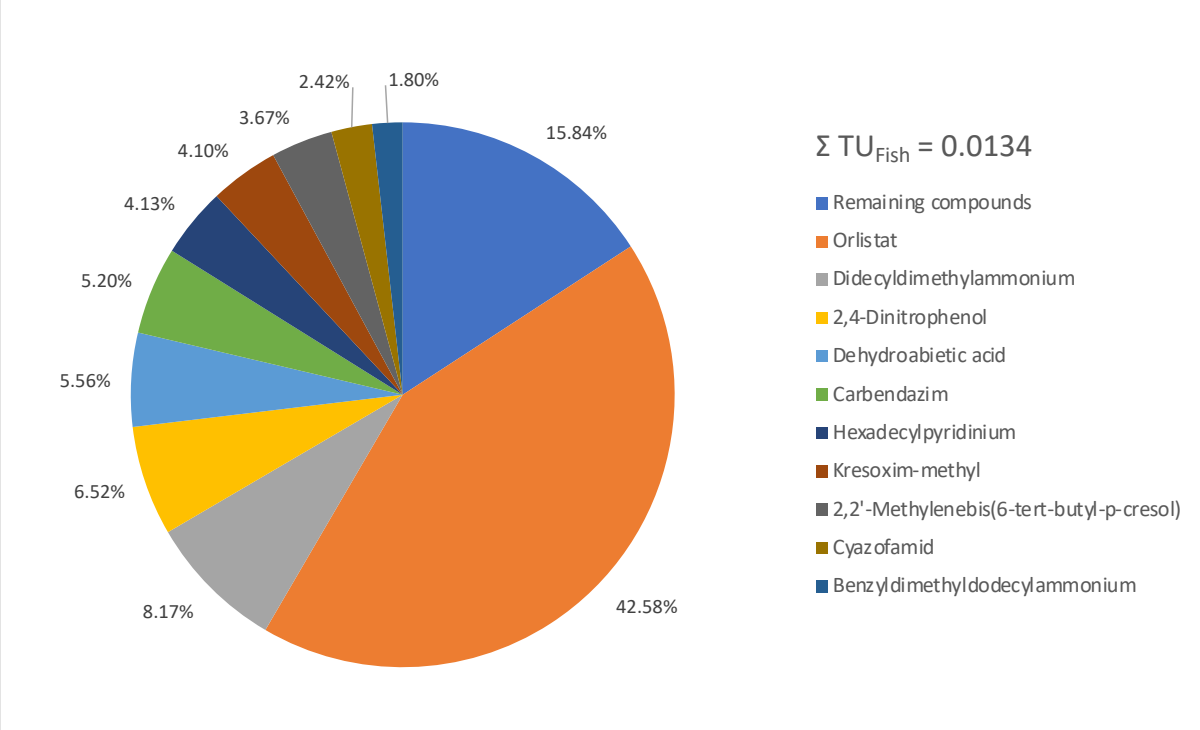
D.13 Lockwitzbach toxicity drivers baseline TU_{Fish}



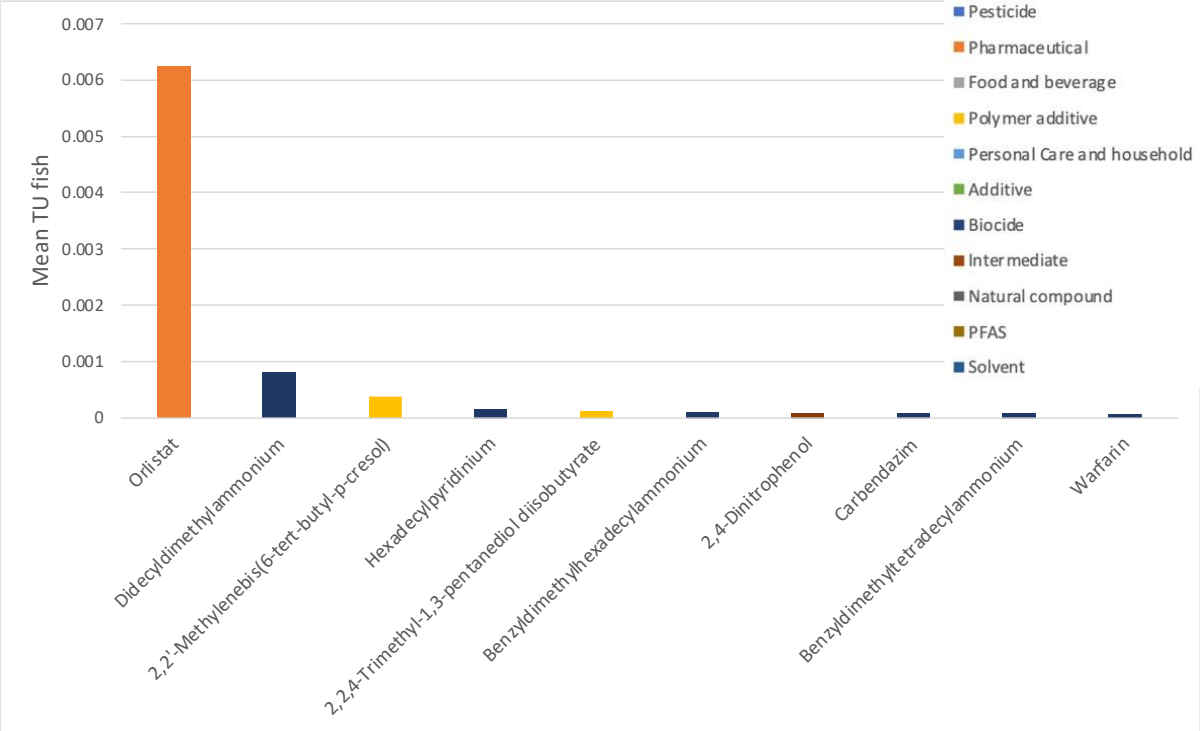


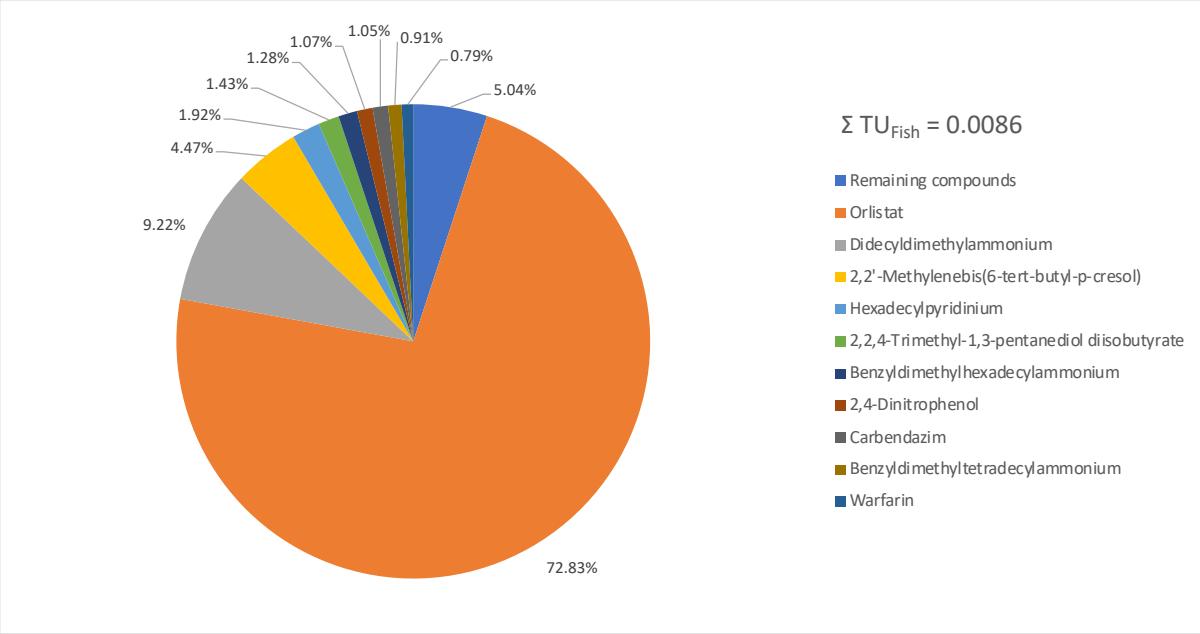
D.14 Lockwitzbach toxicity drivers event TU_{Fish}



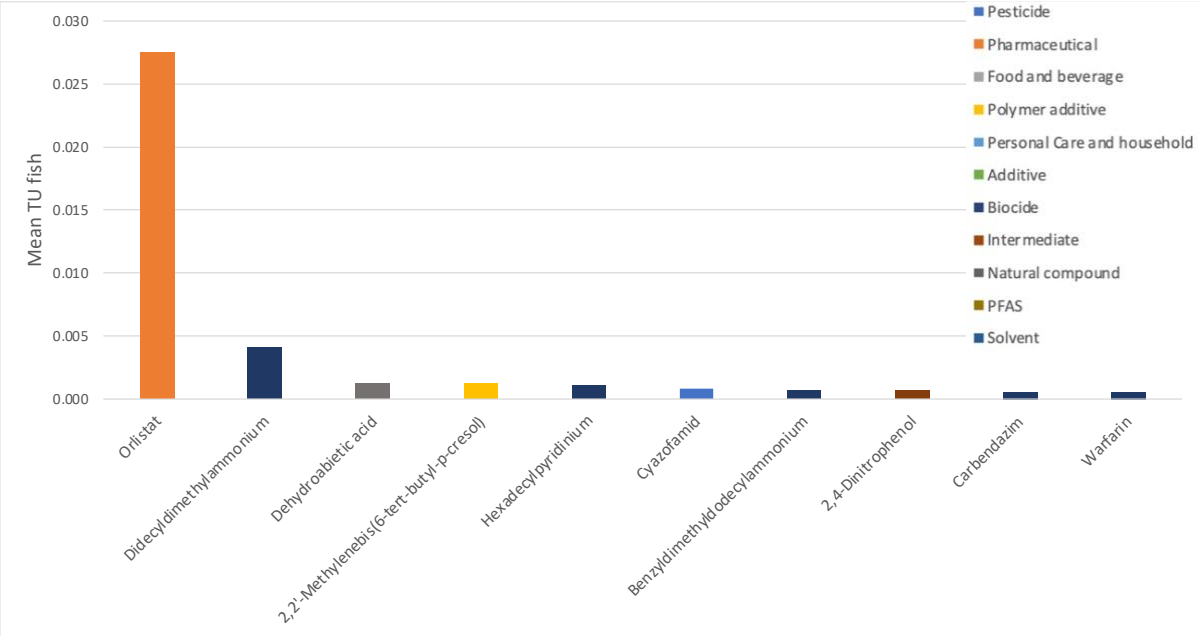


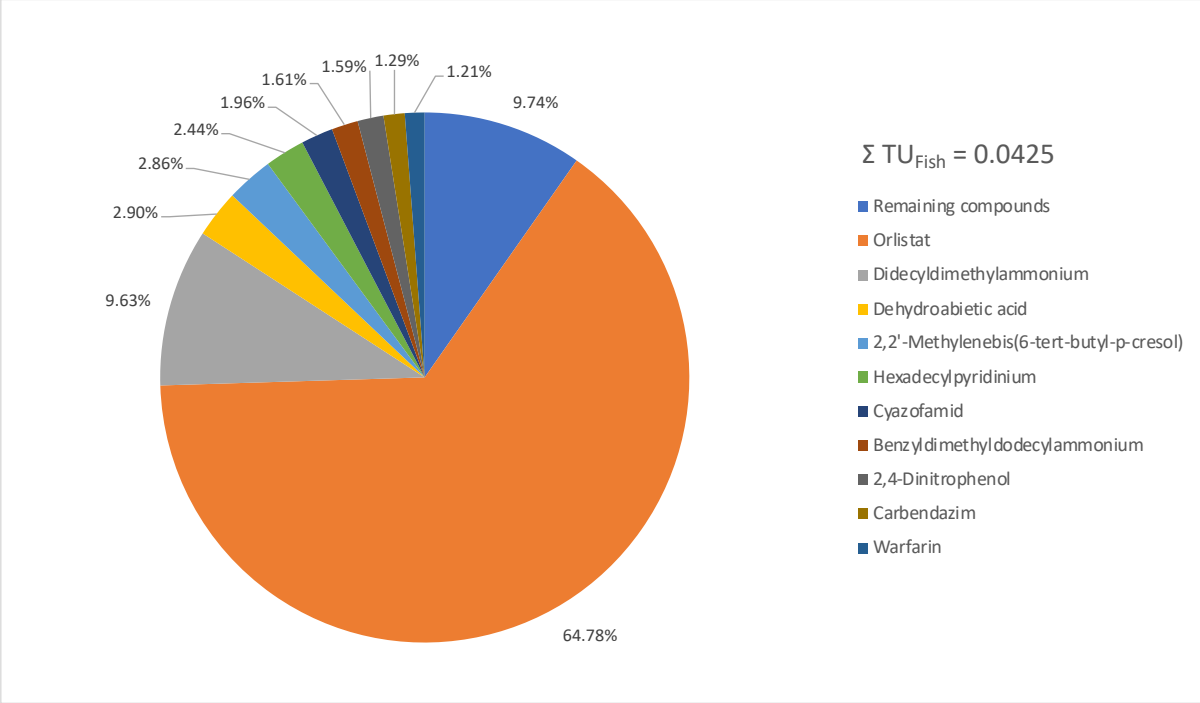
D.15 Geberbach toxicity drivers baseline TU_{Fish}



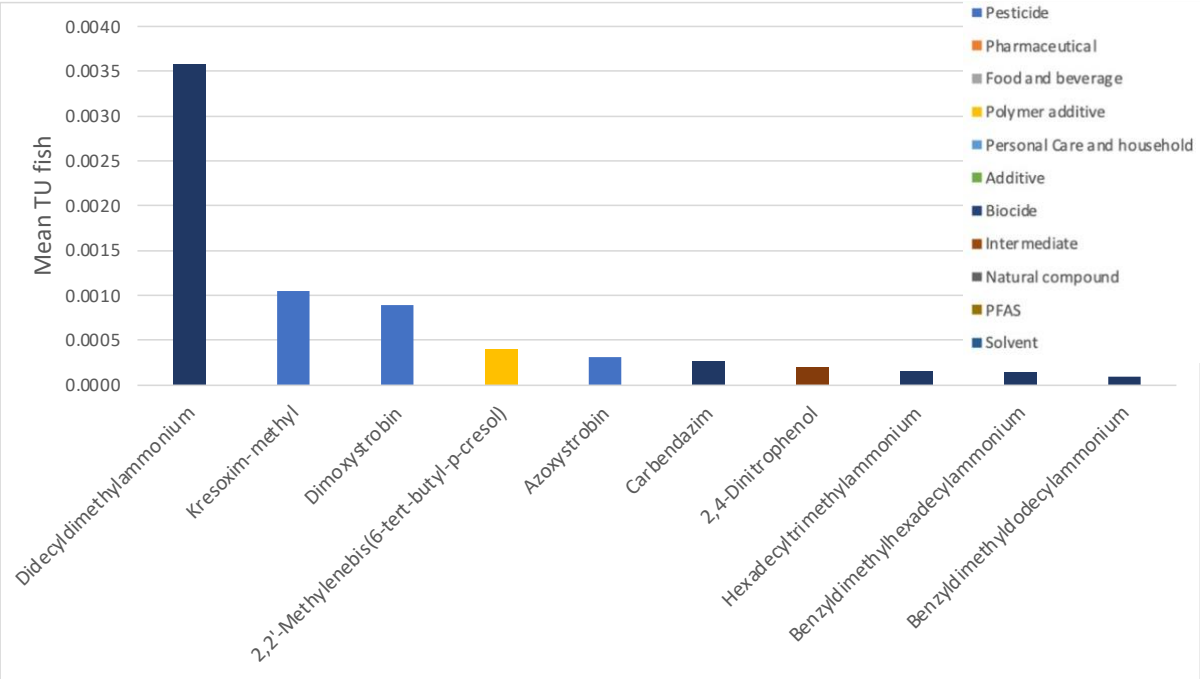


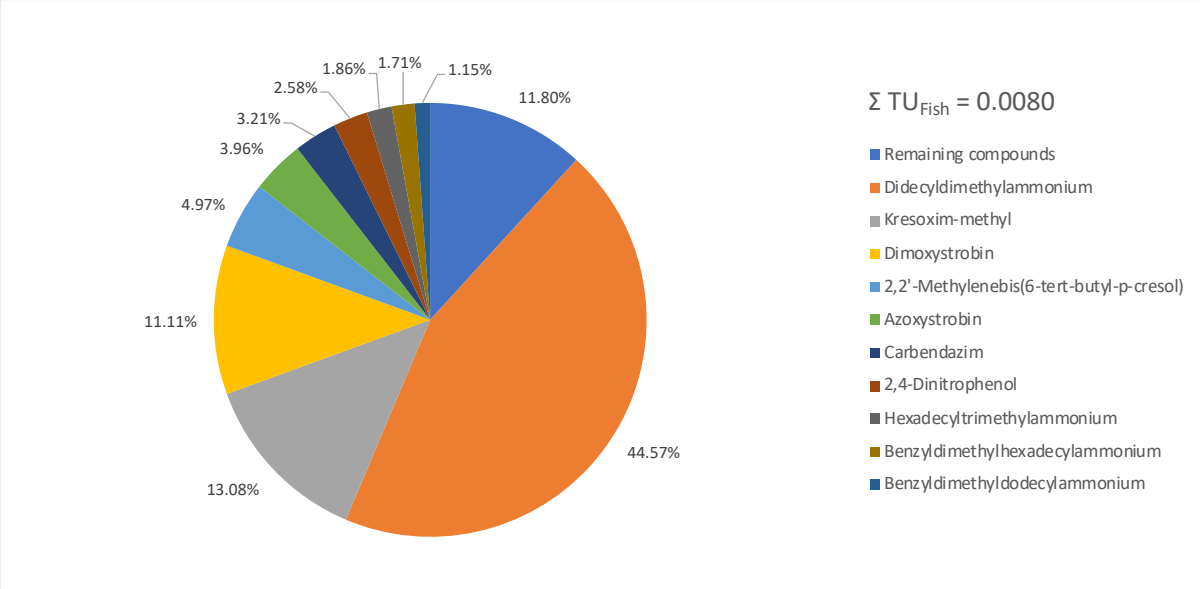
D.16 Geberbach toxicity drivers event TU_{Fish}





D.17 Launzige toxicity drivers baseline TU_{Fish}





D.18 Launzige toxicity drivers event TU_{Fish}